GENETIC INFLUENCES ON APPETITE AND WEIGHT IN INFANCY

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DECLARATION

I, Clare Llewellyn, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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ABSTRACT

The aim of this thesis is to test one of the assumptions of the behavioural susceptibility model of weight that inherited differences in appetite are already present in infancy, and that shared genetic effects are contributing to associations with weight from very early on in life. Data from a British birth cohort of 2402 families with infant twins (Gemini) were used to explore associations between appetite and weight, assess genetic influences on appetite, and examine shared genetic pathways underlying appetite and weight. Study 1 describes the development of a parent-report psychometric measure of infant appetite during the period of exclusive milk-feeding. Four underlying dimensions were identified – 'enjoyment of food' (EF), 'food responsiveness' (FR), 'slowness in eating' (SE), 'satiety responsiveness' (SR), along with a single general item that correlated with all traits ('appetite size', AS). Study 2 established that all traits were significantly associated with higher weight at 3 months and greater increase in weight from birth to 3 months. Study 3 used the twin design to demonstrate moderate to high heritability for all traits (EF: 83%; FR: 59%; SE: 84%; SR: 72%; AS: 77%). Study 4 showed common genetic influence on EF, SE and SR, which explained 78% of the covariation between them, and Study 5 demonstrated common genetic influence between the two satiety-related traits (SE and SR) and weight. Finally, Study 6 was an in-depth exploration of a single case of an infant with extreme appetitive avidity whose parents were forced to exert drastic control measures to avoid severe overeating. This thesis provides evidence for a behavioural susceptibility model of weight because inherited individual differences in appetite are present from early infancy, are phenotypically associated with weight, and share common genetic pathways with weight. Inherited differential susceptibility to the obesogenic environment may be contributing to variability in childhood adiposity.

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CHAPTER 1. EATING BEHAVIOUR AND WEIGHT IN CHILDREN AND INFANTS: A REVIEW OF THE LITERATURE[1]

1.1. The problem of childhood obesity

Obesity is an increasingly prevalent condition that is developing progressively earlier in childhood. UK national statistics (the Health Survey for England, 2007) indicate that in 2004, 14% of 2 to 10 year-olds were obese; almost three times the number in 1990 (The Information Centre, 2008). New trend data has suggested that these numbers will rise even further (Stamatakis et al., 2010b). Until recently the prevailing view was that childhood weight was uninformative about adult weight, partly due to the rarity of childhood obesity (few obese adults had been obese children), and in the light of the fact that many 'chubby' toddlers matured into slim children. However, studies that have followed adiposity trajectories using continuous rather than categorical measures have found strong tracking of weight from early childhood (Lake et al., 1997; Trudeau et al., 2003); and obese children are in fact far more likely to be obese adults than their normal-weight peers (Fuentes et al., 2003; McTigue et al., 2002). Moreover, in recent years there has been a strong interest in the importance of very early life experiences, and it appears to be the case that overweight and obesity in childhood are strongly predicted by rapid weight gain in the early postnatal period (Baird et al., 2005; Ekelund et al., 2007; Ong, 2006), suggesting that causal processes begin soon after birth.

The consequences of childhood obesity for paediatric health include adverse psychosocial effects (Puhl & Brownell, 2001) as well as raised risks of asthma (Chinn et al., 2006) and type II diabetes (Haines et al., 2007). Because obese children are likely to be obese as adults, all of the risks linked with adult obesity, including cardiovascular disease, stroke, type II diabetes, arthritis, and some cancers are also raised (Goran et al., 2003). In response to this, the WHO European Ministerial Conference on Counteracting Obesity (World Health Organisation, 2006) announced their commitment in 2006 to reverse this

[1] Much of the information in this chapter has now been published in a book chapter [Llewellyn C, Carnell S and Wardle J. (2011). Eating Behaviour and Weight in Children. In (Eds) L Moreno, I Pigeot, W Ahrens. *Epidemiology of Obesity in Children and Adolescents (Book I of II) - Prevalence and Aetiology*. Springer series; New York]. During my PhD I have worked on a number of papers, and presented some of my work at international conferences. A list of the papers I have worked on and the conferences I have presented at and attended are shown in Appendix 8.

trend for children by 2015. They underlined the need to develop evidence-based interventions for the treatment and prevention of the disease. A good understanding of the aetiology of childhood obesity is a very important part of the evidence-base.

1.2. The role of eating behaviours and appetite in weight gain

The idea that there might be certain eating styles or appetitive traits that influence weight is not new[2]. In 1968, Stanley Schachter published a seminal paper proposing the 'externality theory' of obesity (Schachter, 1968). It described a series of innovative experiments in which the eating behaviour of a clinical sample of severely obese individuals was compared with the eating behaviour of normal-weight individuals, using a variety of physiological and environmental manipulations. The conclusion was that the obese were more reactive to external cues of food (such as smell or taste) and less responsive to internal physiological sensations related to hunger and satiety, indicating a weakening of normal appetitive controls. In modern environments where highly palatable food is abundant and cheap, high external responsiveness could lead to overeating and weight gain, especially if it is not buffered by strong satiety sensitivity.

Another focus of early research was how emotions differentially influence eating behaviour in the obese and the normal-weight. Focusing again on clinical populations, Kaplan and Kaplan (1957) put forward the 'psychosomatic theory' of obesity, which proposed that obese individuals were not able to discriminate between the physiological arousal caused by emotional states and hunger, possibly because of classical conditioning during early life (Bruch, 1964). This was hypothesised to lead to overeating in response to negative emotional arousal. Schachter, Goldman & Gordon (1968) took a slightly different point of view, suggesting that while normal-weight individuals eat less in response to negative emotional states, the eating activity of the obese remains unaffected. But both theories proposed that differences in food intake between the obese and the normal-weight would be stronger in states of emotional arousal.

[2] Throughout this thesis 'eating behaviours', 'appetite' and 'appetitive traits' are used interchangeably to refer to eating-related characteristics indicative of appetite avidity or food interest.

The paediatric obesity literature developed against this backdrop of research with adults, and likewise focused largely on the clinically obese, but drew additionally upon clinical research with children who 'fail to thrive' (the underweight population). Research into the notion of 'externality' has continued, with attempts to unpick the dimensions of this trait and understand the specific conditions under which children overeat. In particular, Fisher and Birch (1999) explored the notion of 'external' eating by assessing the tendency of children to overeat when presented with palatable foods under conditions of satiety (so-called 'eating in the absence of hunger'); the role of differences in internal satiety sensitivity between overweight and normal-weight children has also been explored using behavioural manipulations (Johnson & Birch, 1994). Another important line of work has examined whether some children 'value' food more highly than others, based on their choice of palatable food over enjoyable activities, given access to both (Temple et al., 2008). Researchers have also been interested in identifying weight-related differences in eating speed, thought to indicate motivation to eat (Llewellyn et al., 2008).

Interest in emotional overeating has continued as well, but paediatric research has also highlighted the tendency of many children to eat less in response to negative emotions (which may represent a natural biological response to stress, as gut activity is inhibited under heightened emotional states (Wardle & Gibson, 2001)), suggesting that this may be a trait that protects against overweight, while emotional overeating may increase the risk of weight gain (Wardle et al., 2001b).

Other behaviours associated with underweight that have also been of interest include the idea that excessive fussiness or pickiness about food may protect against overweight by reducing the number of foods a child is willing to eat (Wright & Birks, 2000). On the other hand, preferences for energy-dense foods (high in the relative number of calories per gram and usually high in sugar and fat) may increase the risk of overweight. Evidence that a high-fat diet is involved in the development and maintenance of overweight has led to efforts to understand food choices (e.g. Hill et al., 1992). It is generally agreed that food choice is significantly influenced by food preferences (Bere & Klepp, 2004; Raynor et al., 2004), making this another area that researchers have been interested in investigating with regard to weight gain.

1.3. Methods and measures used in eating behaviour research with children

As with most behavioural phenomena, a multiplicity of methods have been used to measure eating behaviour in children. Both qualitative and quantitative methods have been utilised, with the latter including behavioural and psychometric quantification, while the qualitative work tends to be based on clinical observations or interviews with severely obese individuals or their mothers, or with mothers of children who are 'failing to thrive'. The main quantitative and qualitative methods that have been developed are described in more detail in the following sections.

1.3.1. Qualitative assessment of eating behaviour

Not surprisingly, qualitative methods such as clinical interviews have predominated in clinical research. For example, much of the early work that led to the development of the 'Psychosomatic Theory' of obesity evolved from the field of psychosomatic medicine where investigators explored the idea that obesity was the result of psychopathology. This involved characterisation of the psychopathological profiles of the obese population through clinical interviews within a therapeutic context. Likewise in the paediatric literature, clinical observations and interviews were the method of choice for understanding disordered eating patterns among clinically underweight children. Insights from these methods highlighted excessive fussiness or pickiness about food and emotional under-eating as defining features of this population of children (Harris, 1993). While qualitative methods are useful for the initial characterisation of particular eating styles within well-defined groups, quantitative methods are needed to test hypotheses that certain eating behaviours are systematically related to weight.

1.3.2. Quantitative behavioural measurement of eating behaviours in children

The researchers who developed 'externality theory' in the adult population drew heavily on experimental behavioural work, and paediatric studies have followed suit. A number of different behavioural paradigms have been developed to quantify sensitivity to internal satiety mechanisms and responsiveness to external cues of food, as well as food preferences.

1.3.2.1. Energy compensation

The 'energy compensation' paradigm tests how responsive individuals are to internal cues of satiety. The method assumes that if individuals are given a 'preload' of food (or liquid) prior to eating a meal, those with good internal response mechanisms will down-regulate food intake at the meal in proportion to the amount of energy consumed in the preload. Typically the preload varies along a continuum of calories, and is carried out over two or more testing sessions. Each participant receives a compensation score (COMPX) indicating how much their meal intake compensated for the preload using a standardised formula ((Ad-libitum intake $KJ_{low\ energy\ preload}$ – Ad-libitum intake $KJ_{high\ energy\ preload}$) / (Drink preload KJ_{high} – Drink preload KJ_{low} x 100%) (Johnson & Birch, 1994)). Studies that have explored compensation ability in children have found large individual differences (Tanofsky-Kraff et al., 2007).

1.3.2.2. Microstructural analysis of ingestive patterns

Microstructural analysis of ingestive patterns involves observing a meal being eaten and characterising the behaviour in detail in smaller structures such as number of bites or quantity of food per unit of time (e.g. bites/minute). This allows for within-meal and between-participant comparisons of behaviours throughout the course of the meal (Guss & Kissileff, 2000). An eating rate that slows down, as characterised by a deceleration curve, is assumed to reflect a 'normal', biologically-determined satiation process, while non-deceleration has been hypothesised to indicate impaired satiety responsiveness (Meyer & Pudel, 1972). The average rate at which food is consumed throughout the meal is thought to indicate hunger level or motivation to eat, with a faster eating rate also compromising satiety by outpacing the physiological control mechanisms.

The microstructure of infant feeding can also be assessed by studying sucking behaviour. During the early 1960s Kron and colleagues (1963) designed apparatus to measure a number of facets of sucking behaviour in young milk-feeding infants in a laboratory, to allow for detailed inspection of this distinctive early life behaviour. The infant sucks on a rubber nipple within which a sensor measures the pressure of each suck, and the flow rate of the milk into the nipple for each suck; the output includes summary data such as the average pressure of each suck, the rate of sucking (e.g. number of sucks per minute), and

the volume of milk consumed per suck and in total (Agras et al., 1987). In a subsequent validation paper relating to the equipment Kron and colleagues (1968) reported that there were considerable individual differences in all of the variables measured by the equipment that were observable during the first 4 days after birth. Moreover, the individual differences appeared to be consistent across 18 observed feeds. Importantly, the different aspects of feeding that were measured were correlated – infants who fed at a faster rate tended also to suck with greater vigour and consumed more milk during an average feed (Kron et al., 1963), suggesting that these variables are all tapping aspects of appetite avidity akin to those measured using microstructural analysis in adults. To support this, using similar equipment Dubignon and Cooper (1980) were able to show that 1 week old infants who were classified as 'poor feeders' (milk intake <25th percentile) fed considerably more slowly and sucked much less often than 'good feeders' (milk intake >75th percentile). Interestingly, infants appear to demonstrate the same pattern of sucking deceleration (the natural satiation curve) over the course of the feed as that observed in adults – during the last quarter of a feed, Dubignon and Campbell (1969) observed that infants spent significantly less time sucking, and made fewer sucks than the earlier quarters of the meal.

Devices have also been developed to characterise the sucking behaviour of breastfeeding infants. One option is to put an electronic strain gauge under the infant's chin (deMonterice et al., 1992; Ramsay & Gisel, 1996); a less intrusive method is to observe the behaviour directly and code it as it occurs or subsequently using video footage (Drewett & Woolridge, 1979; Kaye, 1977).

1.3.2.3. Sensory activation of eating

The 'sensory activation of eating' involves exposing children to a number of different sensory food cues (e.g. smell and taste of highly palatable foods) versus no food cues (control task), and assessing how much of that same food is consumed following exposure (Jansen et al., 2003). It is hypothesised that hyper-responsive children will consume more of the palatable food if they are exposed to the sensitising properties of it (e.g. sight, smell or taste), than in the control condition. This method has also been adapted to explore how milk-fed infants respond to milk or to other liquid foods of varying palatability. One option is to measure changes in sucking behaviour (e.g. sucking pressure, sucking rate, or total amount consumed) from one fluid type to the next, or between groups (Crook, 1978;

Crook, 1977). For example, flavouring breast-milk with vanilla led to increased feed length and total amount consumed (Mennella & Beauchamp, 1996), suggesting that the improved palatability increased milk consumption across the board.

1.3.2.4. Eating in the absence of hunger

'Eating in the absence of hunger' (EAH) measures intake of highly palatable foods under conditions of satiety, and is assumed to indicate the extent to which palatable foods override internal satiety regulation, thereby tapping responsiveness to external cues to eat. Typically, a child will be fed a mixed meal and instructed to eat until full. Following the meal each child is tested individually and tastes and rates a variety of snack foods, after which they are left alone with free access to the snacks and toys and intake is assessed (Fisher & Birch, 1999). In order to exclude the confounding effects of hunger on intake, children are sometimes asked to rate their hunger level following the meal and those who are still hungry are excluded from the analyses, although a limitation of this method is uncertainty about the reliability of this information in children. One drawback of the EAH paradigm is that subsequent intake of palatable foods may reflect both hypersensitivity to external food cues and insensitivity to internal satiety cues. Nevertheless, this behaviour appears to be fairly stable over a two-year period. Although all children on the whole have a tendency to become more food responsive as they get older (i.e. as a general rule children tend to eat more in the absence of hunger with increasing age), they tend to keep their relative position among their peers – to demonstrate this Fisher and Birch showed that 64% of girls who were categorised in the lowest half of scorers at age 5 were also in this category at age 7, and 68% of those who were in the highest half were also in the high scoring group two years later (Fisher & Birch, 2002).

1.3.2.5. Behavioural economic analysis of food choice

Individual differences in the level of pleasure derived from eating highly palatable foods may drive motivation to eat those foods. This can be explored by assessing the 'relative reinforcing value' (RRV) of food (Lappalainen & Epstein, 1990). This measures the amount of effort that an individual is willing to make to obtain food of higher versus lower palatability, or food versus non-food rewards, and is thought to tap an individual's

motivation level to eat that food. Concurrent schedules of reinforcement are set up such that the work requirement to obtain the desirable food becomes progressively more effortful, while the non-food (or lower-palatability food) schedule remains constant (Lappalainen & Epstein, 1990). The point at which the child opts for the alternative reward over the palatable food reward provides an index of the subjective (maximum) value placed on the desirable food. The harder an individual is prepared to work for the desirable food, the more food responsive they are deemed to be, and the higher they value food over other pleasurable activities.

A related concept is 'habituation' to food offerings (of the same or different foods). 'Habituation' is the process by which an individual's attention or interest in a stimulus wanes over repeated presentations of it (Groves & Thompson, 1970). It is a well-known phenomenon across a wide range of domains, and a slower rate of habituation to food has been shown to lead to a larger consumption of food in experimental settings (Wisniewski et al., 1992). 'Habituation' to food can be measured using a similar paradigm to that of the reinforcing value of food – rate of habituation is characterised by the rate of decrement in responses over the course of the schedules (e.g. Epstein et al., 2008).

1.3.2.6. Food preference studies

Typically, behavioural assessment of food preferences involves the children taking part in a taste test in which they sample a range of foods and then rank them in order of preference or rate them (e.g. Fisher & Birch, 1995). Birch (1979) has developed an assessment method whereby children can indicate their preferred food using cartoon faces that are happy, neutral or unhappy. Food preference data collected using this method has been shown to be reliable in young children (Birch, 1979). Other methods involve using questionnaires that ask parents or children to rate their liking of a variety of food types to create preferences scores for groups of foods.

1.3.2.7. Direct observation of feeding patterns of children or infants who 'fail to thrive'

Much of the research within the field of 'failure to thrive' has involved direct observation of infant or child eating behaviour during 'typical' meals to identify qualitative behavioural

differences between those who are gaining insufficient weight and those who are growing 'normally'. Studies tend to videotape the eating episode and subsequently code behaviour (e.g. Chatoor et al., 2001) or use milk-feeding diaries to assess intake in infants.

1.3.2.8. Limitations of behavioural studies of eating in children

While observational studies of behaviour are often regarded as the gold standard in that they provide objective measures of eating behaviour, they are not always practical, and can be very expensive to organise on the scale necessary for adequate statistical power to detect small relationships with weight. Furthermore, it is possible (and even likely with some samples) that children modify their eating behaviour because they are aware that they are being observed; this may be compounded by unfamiliar experimental settings such as research laboratories. In addition, due to research costs and practicalities many studies are only able to observe a single meal or episode of the behaviour of interest which limits the possibility of drawing inferences about the general trait underlying the measurement paradigm (Epstein, 1983). Another consideration is that a single measurement of the behaviour is susceptible to random factors at play at the time of assessment, introducing 'noise' and reducing the power to detect relationships (Carnell & Wardle, 2007).

1.3.3. Quantitative psychometric measurement of eating behaviours in children

Psychometric measures (standardised quantitative questionnaires) lose the objectivity of behavioural assessment but have the advantage of reflecting behavioural characteristics aggregated over many situations in contrast to the 'snapshot' provided by a single test meal. Respondents are asked to give answers that typify their behaviour or attitudes in general, reducing the opportunity for intrusion by unusual instances or deviations from the 'norm' (Wardle et al., 2001b). Other advantages are that they are inexpensive and easy to distribute, so can be administered on a large scale, maximising statistical power.

However, self-report measures of behaviour can be problematic for children who are limited developmentally; it is unlikely that young children will adequately understand the

questions, or have sufficient self-awareness to provide accurate information about their own behaviour. An alternative is to use a parent-report questionnaire for younger children (Wardle et al., 2001b). Although parent-reports of behaviour are necessarily subjective, and may reflect socially desirable responses (especially from parents with overweight or obese children), parents hold a unique position in that they have the opportunity to observe their children regularly in their natural environment, arguably making them the most accurate informants of their child's behavioural traits (Carnell & Wardle, 2007).

A number of studies have used modified versions of adult self-report questionnaires in studies with children, usually to detect eating disorder symptomatology (e.g. Lluch et al., 2000; Shunk & Birch, 2004; Snoek et al., 2007). One of these questionnaires is the Three Factor Eating Questionnaire [TEFQ] (Stunkard & Messick, 1985) which measures dietary 'restraint' (deliberate suppression of eating in order to control weight), 'disinhibition' (episodes of perceived lack of control over eating in response to certain emotional and situational cues) and 'hunger' (pervasive and persistent feelings of hunger and cravings); 'disinhibition' and 'hunger' tap aspects of external eating but the TFEQ has only been used with adults and older adolescents (e.g. de Lauzon et al., 2004). The Dutch Eating Behaviour Questionnaire [DEBQ] (van Strien et al., 1986) which measures 'external eating' (eating in response to external food cues such as the sight or smell of palatable food), 'emotional eating' (the tendency to overeat if experiencing negative emotional arousal) and 'restrained eating' (deliberate suppression of eating) has been used far more extensively with children. Some studies have used the adult self-report version with children (e.g. Lluch et al., 2000; Shunk & Birch, 2004; Snoek et al., 2007), but a parent-report version of the DEBQ also exists [DEBQ-P] (Caccialanza et al., 2004), as does a child self-report version which has been validated for use in 7-12 year olds [DEBQ-C] (van Strien & Oosterveld, 2008).

The most recent development is the Child Eating Behaviour Questionnaire [CEBQ], designed to assess the main eating styles implicated in the development of both under-weight and overweight (Wardle et al., 2001b). Scales include behaviours that have typically been of interest in the 'failure to thrive' literature such as 'fussiness about food' and 'emotional under-eating', as well as scales that assess the eating styles associated with overweight and obesity in the behavioural literature, such as 'satiety responsiveness' (akin to sensitivity to internal satiety mechanisms), 'slowness in eating' (similar to eating

rate measured using microstructural analysis of ingestive patterns), 'food responsiveness' and 'enjoyment of food' (both similar to the behavioural measures of responsiveness to external cues of food). The eating styles represented by these scales may be categorised into 'food approach' behaviours which indicate higher appetitive responses (such as 'food responsiveness' and 'enjoyment of food'), 'food avoidance' behaviours which indicate better appetitive control or less interest in food (such as 'satiety responsiveness', 'slowness in eating' and 'food fussiness'), and 'emotional eating'. The CEBQ has been validated using behavioural measures and the scales correlated well with the behavioural measures on which they were based (Carnell & Wardle, 2007). Moreover, although scores on all scales appear to change over time from 4 to 11 years with all children becoming more 'appetitive' across the board as they get older, children who score relatively highly on a scale at age 4 also score relatively highly on the same scale at 11 years, so children keep their relative position indicating trait stability (Ashcroft et al., 2008).

1.4. Current evidence relating eating behaviours to weight in children

1.4.1. Assessing the relationship between eating behaviour and weight in children

Most studies assessing how eating behaviour relates to weight have utilised case control designs, comparing the behaviours in normal-weight versus overweight or obese children (or those who are 'failing to thrive'). Furthermore, many studies, especially the earlier ones, have utilised clinical samples of severely obese individuals. Two limitations of case control designs should be considered. Firstly, eating behaviours may be modified by the individual as a result of having been classified clinically as overweight or obese – for example some individuals may be using behavioural techniques in an attempt to lose weight, masking the true expression of underlying traits (Carnell & Wardle, 2008b). Although this may be less of an issue among young children, parent-report measures may still be associated with bias – parents of very overweight children may be overly influenced by their child's weight status when answering questions about their appetite, assigning greater appetite avidity to the child in order to explain their weight struggle. Secondly, adiposity is approximately normally distributed and the overweight and obese categories are essentially arbitrary cut-offs toward the upper end of the adiposity continuum; moreover, overweight and obesity no longer represent rare cases, but rather the

population as a whole is gaining weight (Carnell & Wardle, 2008b). However, a good theory of the aetiology of weight should be able to explain variation in adiposity across the entire spectrum, not just distinguish the categories of 'overweight' and 'obese' from 'normal-weight'. It is also of considerable importance that individuals who sit at the upper end of the 'normal-weight' range are at higher risk of weight-related health issues than leaner individuals, highlighting the need to understand weight at all levels.

Even after taking into account the issues pertaining to sampling or study designs, there are other obstacles to identifying eating behaviours that are implicated in the development and maintenance of overweight. Weight gain is in many cases the result of tiny aberrations in energy balance compounded over several years. An excess of 100kcal per day is sufficient to add over 5kg of fat over 1 year assuming energy is stored at 50% efficiency and an excess of 3500 kcal leads to a net gain of about 1 pound of fat (Hill et al., 2003). Identifying the behaviours that explain such small differences in intake is a challenge, and measurement error may obscure such marginal effects. All of these considerations should be borne in mind when reviewing the evidence below (the literature reviewed below has been summarised in tables that may be viewed in Appendix 1).

1.4.2. Responsiveness to food cues[3]

1.4.2.1. Sensory activation of eating

Using a 'sensory activation of eating' approach, Jansen et al. (2003) compared the intake of highly palatable snack foods by obese and normal-weight children (*n*=31) aged 8-12 years, in three conditions each one week apart. In two different exposure conditions, intake of a number of appetising snack foods was measured after i) consumption ('preload') of a small amount of each of the palatable snack foods, and ii) after the intense smelling of the snack foods ('smelling'); in the third control condition intake was measured following a 10-minute non-food activity. They hypothesised that obese children are stimulated more by food cues so would eat more after intensive exposure. In support of this hypothesis, the obese children showed a stronger behavioural reaction to exposure to food cues (smelling), with a greater difference in intake between control and exposure

[3] See Appendix 1.1 for a summary table of the literature.

('smelling'), whereas the normal-weight children actually ate less than in the control condition.

Another research group has used this approach to explore if overweight and obese children consume more snack foods than leaner controls following exposure to food adverts (Halford et al., 2004; Halford et al., 2007b; Halford et al., 2008). In the first study, 9-11 year old children (*n*=42) were exposed to 10 minutes of non-food adverts and food adverts, two weeks apart; immediately following the viewing the children were invited to eat a variety of low-fat and high-fat snack foods (Halford et al., 2004). There was a main effect for weight group in that overweight and obese children consumed more than normal-weight controls regardless of condition, and the effect appeared to be graded. In addition, all children consumed significantly more following exposure to food adverts, although the effect was slightly more pronounced for the overweight and obese children. The research group repeated this experiment again with the same age group (*n*=59), but including a larger number of overweight and obese children to examine weight status differences more reliably (Halford et al., 2008). In the second replication they found again that all children consumed more following exposure to food adverts, but this time there was a much clearer effect of weight status on intake – no effects of weight group were observed for intake following non-food adverts, but there was a significant difference between the groups in intake in the food adverts condition with obese children consuming significantly more than both the overweight and normal-weight groups, and the pattern of intake again appeared to be graded.

The study was repeated with 5-7 year olds to ascertain if these weight group effects are present earlier in life (*n*=93). No effect of weight group was found, although a small but significant association (0.19) was observed between BMI and intake following exposure to food adverts (Halford et al., 2007b), suggesting that a small effect may be present earlier in life but that this trait becomes exaggerated over time. It may be the case that this trait becomes more ingrained with experience – as children become more independent and accustomed to both watching television and helping themselves to snack foods more freely, this characteristic becomes reinforced.

Responsiveness to the sensory properties of food has also been evaluated in infants at higher or lower risk of obesity, according to their parental weight status. Milstein (1980)

found that babies with two overweight parents sucked more avidly in response to a sweetened solution compared with plain water, than did babies with two normal-weight parents.

1.4.2.2. Eating in the absence of hunger

A number of studies using the EAH paradigm have also provided evidence in support of the externality theory, although the results indicate that many psychological and social factors may also play an important role in influencing the expression of this trait. One of the earliest studies of this kind was conducted by Fisher and Birch (2002) in a laboratory setting. They found intake of palatable snack foods to be higher in overweight girls compared with normal-weight girls (*n*=165-181) at age 5 and at 7 years (Fisher & Birch, 2002), and this finding was replicated in a much larger sample of girls and boys ranging from 5-18 years (*n*=725) also in a laboratory testing situation (Fisher et al., 2007). A very recent study by Shomaker and colleagues (2010) explored whether the extent of EAH was influenced by the size of the meal given beforehand (a 'large array' buffet meal of >10,000 kcal and a 'standardised meal' matched for 50% of each participant's energy requirements), in a laboratory setting in 13-17 year old adolescents (*n*=78). They found that EAH intake was slightly smaller after the larger meal across the board, but that overweight adolescents had higher EAH scores than normal-weight participants overall; BMI was also associated with EAH in the continuous analyses, after adjustment for meal size, supporting the findings of the two previous studies.

Fisher and Birch's research team were also interested in exploring this phenomenon in the context of obesity risk (indexed by parental weight status). In keeping with the earlier findings they found that girls at higher risk of obesity (by virtue of both parents being overweight) demonstrated a more potent response (i.e. consumed significantly more) than girls at lower risk (those who had only one overweight parent or none) at 7, 9, 11 and 13 years of age (*n*=168-197) (Francis et al., 2007). Moreover, although all of the girls increased their scores from age 7 to age 13, the highest risk girls showed a much greater increase in EAH than the other groups, characterised by a statistical interaction between age and risk group, and this was accompanied by comparatively greater increases in BMI over the same period (Francis et al., 2007). Similar results were obtained only looking at

maternal weight status from 5 to 9 years (Francis & Birch, 2005), and using the girls' actual weight status (Shunk & Birch, 2004).These findings suggest that this trait may be one of the mechanisms through which these girls ultimately attain their projected overweight status.

If this were the case, one would expect EAH scores to predict weight gain over time. This was not supported in a study that explored the role of EAH in weight gain over a one year period using the same large sample (*n*=798) of 4-19 year old males and females as Fisher and colleagues (2007), although one year may not be long enough to show the weight gain sequelae from this trait under normal circumstances (Butte et al., 2007). On the other hand, a very early study did find a longitudinal association using an insightful observational method; during the first week of an 8-week long summer camp researchers covertly measured how many M&Ms 9-15 year old children (*n*=92) ate immediately following breakfast, lunch or dinner and assessed weight during the first and last weeks of the stay (Rodin & Slochower, 1976). In contrast to the previously cited study, the authors found a significant difference in weight gain by baseline levels of EAH despite the substantially shorter time period and much smaller sample size. It is possible that this significant finding resulted from children being given free access to food over the 8-week summer camp, which, compared with their usual home environment, may have constituted a substantial decrease in food restriction allowing this trait to be expressed most vehemently so that it might have the opportunity to lead to weight gain.

The relationship between EAH and weight was also assessed using community samples in two naturalistic settings very recently – within a school with 7-9 year olds (*n*=348), and at home with 9-12 year old twin children (*n*=316). For the first time, these two studies examined the nature of the relationship between EAH and adiposity across the full spectrum of weight. In both samples EAH was positively and linearly associated with BMI for boys, but the association was weaker for girls (Hill et al., 2008). Interestingly in the study that used school-based testing, where the task was carried out collectively within the classroom, girls demonstrated progressively higher EAH from underweight through lower and higher healthy weight, but there was a slight decline within the overweight and obese range; in the other study in which the task was completed by sibling pairs at home, girls showed a graded increase in EAH across the entire range, although it did not reach significance. The context of the testing may have played an important role in the different

findings – overweight and obese girls may behave differently when being observed by their peers at school than they would in the privacy of their own home, in order to manage judgements by others made about their weight status on the basis of their eating behaviour, while being overweight as a boy does not come with the same prejudices so they are less likely to overtly alter their behaviour. This is supported by studies that have found that overweight women experience weight stigmatization more often than overweight men (Cossrow et al., 2001), and that 9-10 and 12-13 year old males from the US are less concerned about their weight, perceive less concern from friends and family about their weight, and are less likely to be attempting to lose weight compared with girls of the same age (Adams et al., 2000).

Two other studies have also reported associations for boys only: Moens and Braet (2007) found that overweight boys demonstrated higher EAH than normal-weight boys aged 7-13 years (*n*=16) in a laboratory testing session, while no difference in intake was demonstrated in girls (*n*=36). Similarly, Faith and colleagues (2006) found that 5-year old boys at higher risk of obesity demonstrated higher EAH than those at lower risk (based upon maternal pre-pregnancy weight) (*n*=27), but no difference was found in girls (*n*=26). As with the study by Hill and colleagues (2008) social desirability bias may help to explain the sex difference observed in Moens and Braet's (2007) study – the overweight children were on a waiting list for a weight loss intervention and the girls may have restricted their intake in an attempt to demonstrate their good intentions. The non-significant finding reported in the study by Faith and colleagues (2006) may result from the very small sample size as well as the young age. The relationship between EAH and risk for overweight is small, considering that weight gain probably results from small differences in this behaviour aggregated over many years, so a sample of only 15 girls at risk for overweight is unlikely to be large enough to reliably find an association. Moreover, this trait appears to become more pronounced as children get older highlighting the importance of an adequate sample size when looking earlier in life (Fisher & Birch, 2002; Francis et al., 2007). Lastly, EAH may be less pronounced in girls than in boys more generally – e.g. Fisher and colleagues (2007) and Hill et al (2008) both found that on the whole boys consumed substantially more than girls 'in the absence of hunger' – further reducing power to detect effects among girls due to lower variability in female versus male data.

To complicate matters further, one study has reported an association between EAH and weight for girls but not for boys, in a sample of 3-6 year olds (*n*=75) who were tested individually in a room set up in the children's school (Cutting et al., 1999). It is clear that more research is needed to assess this eating behaviour in different settings, across the full spectrum of weight, and longitudinally to elucidate when and why this behaviour is expressed in girls and boys.

1.4.2.3. Behavioural economic analysis of food choice

An important group of studies have operationalised responsiveness to food as the reinforcing value of food. They have provided fairly convincing evidence of an association with body size. Studies with adults have suggested that food is more reinforcing for overweight than for normal-weight participants (Epstein et al., 2007; Johnson, 1974; Saelens & Epstein, 1996), and that those adults who 'value' food more highly in this paradigm, consume more food in an ad libitum setting than do their counterparts with a lower reinforcing value for food (Epstein et al., 2004; Epstein et al., 2007).

Only a handful of studies have used this technique to assess the same relationship in children. Temple and colleagues (2008) recently reported on two different food reinforcement studies with groups of overweight and normal-weight children (*n*=45; *n*=45), in each case children were aged 8-12 years and recruited from the local community. In the first study, the researchers compared the reinforcing value of pizza versus a (favourite) non-food activity such as a hand-held video game, colouring or a magazine, in normal-weight and overweight children (*n*=45). The task used a progressive schedule of reinforcement for food (the number of button presses the child was required to make to gain each additional slice of pizza doubled each time) concurrent with a variable ratio schedule for non-food (an average of four button-presses was required throughout the session). The second study compared in normal-weight and overweight children (*n*=45) the reinforcing value of a selection of snack foods and a (favourite) non-food activity, using progressive schedules of reinforcement for both the food and non-food alternatives, which doubled each time in each case. In both studies, the overweight children found food more reinforcing than normal-weight children: in Study 1 the overweight children made significantly more responses for food as the reinforcement schedules progressed

(characterised by a statistical interaction between BMI and reinforcement schedule) and consumed significantly more energy than the normal-weight children; in Study 2 heavier children found food more reinforcing than the non-food alternative whereas the normal-weight children found the alternative activity more reinforcing than food (indicated by a statistical interaction between weight status, reinforcer type and schedule of reinforcement), and likewise the overweight children consumed more than did the normal-weight participants.

In a similar study, the same research group explored the relationship between 'habituation' and weight by comparing in 8-10 year old normal-weight and overweight children (*n*=34) their response rate decline over the course of 10 2-minute (fixed interval) trials during which they 'worked' for access to cheese burger (Temple et al., 2007). They found that the rate of decline in responses for food was slower for overweight children than for their leaner peers over the task period (characterised by an interaction between weight status and trials, indicating that as the task progressed normal-weight children made fewer responses than overweight children), and the overweight group consumed more energy than the normal-weight children. These findings indicate that overweight children may habituate slower to food cues than their leaner counterparts, and that this process is facilitating intake of greater amounts of energy.

This research group subsequently extended their research to include more than one food type and to explore if the 'sensitisation' level of the individual (an increased rate of responding at the very beginning of the task) moderated the relationship between weight status and habituation (Epstein et al., 2008). This time overweight and normal-weight 8-12 years olds (*n*=65) responded for either pizza or macaroni cheese. Overweight children demonstrated less habituation for food across the trials than the normal-weight children, regardless of the food type they were responding for. Moreover, eight out of 30 overweight children did not adequately 'habituate' to the food over the 28 minute task period, compared to only one of the 35 normal-weight children. It was noteworthy that the process of sensitisation seems to play an important role in moderating the relationship between weight status and habituation rate – that is, there was a bigger difference between weight groups for 'sensitisers' than for 'non-sensitisers'. Energy intake over the task was again higher for the overweight children. This study was repeated with another group of children of the same age (*n*=84) using a variety of snack foods or the same snack food, to explore

the role of food variation on habituation in overweight and normal-weight children (Epstein et al., 2009). The findings were the same insofar as overweight children habituated more slowly than normal-weight children (using number of responses made as the outcome measure). On the other hand, there was a significant interaction with food reinforcement type ('same' or 'variety') given on total energy intake which showed that overweight children only consumed more energy than the normal-weight controls if given a variety of foods as the reinforcement, while there was no difference between the weight groups when they were given the same snack food. This study was perhaps more representative of a 'real life' situation as children tend to be confronted with more than one food type to respond to; the findings suggest that the effect of food variety on intake is greater for overweight than for normal-weight children.

Recently the association between the reinforcing value of food and change in adiposity over time was investigated in children aged 7-9 years (*n*=316) using a simplified version of the task designed for use outside a laboratory setting (Hill et al., 2009a). Higher reinforcing value of food at baseline predicted change into a higher weight category over the year, and this was the case for children across all levels of the weight spectrum, suggesting that the reinforcing value of food for an individual child may play a causal role in weight gain.

Collectively, these studies provide convincing evidence that there are motivational differences between normal-weight and overweight children regarding food; overweight children are prepared to work harder to obtain food, consume more of it once it is given, and their interest in food does not wane with repeated exposure to the extent observed in normal-weight children. Most importantly, this drive for food predicts weight gain over a one year period implicating a causal role for individual differences in motivation to obtain palatable food.

1.4.2.4. Psychometric measures of food responsiveness & appetite avidity

Psychometric measures of responsiveness to external cues of food have generally supported the hypothesis that fatter children are more responsive than thinner children (Webber et al., 2009; Viana et al., 2008; Spence et al., 2010; Sleddens et al., 2008;

Parkinson et al., 2010; Joyce & Zimmer-Gembeck, 2009; Jahnke & Warschburger, 2008; Gregory et al., 2010; Cunha et al., 2010; Carnell & Wardle, 2008a), although not all studies have demonstrated the association (Halford et al., 2004; Hill et al., 1994; van Strien & Oosterveld, 2008), which appears to be influenced by the reporter and the sample. In particular, there is reason to believe that child-report measures of external eating are unreliable in comparison to parent-report measures.

For instance three out of five studies identified that assessed food responsiveness in children using a child self-report version of the 'external eating' scale from the DEBQ failed to find a significant relationship with weight (Halford et al., 2004; Hill et al., 1994; van Strien & Oosterveld, 2008). What is more, the other two studies found negative associations such that overweight children with a higher BMI actually reported less 'external eating' than the normal-weight controls (Braet et al., 2008; Wardle et al., 1992), in contrast to the behavioural data, suggestive of measure unreliability. Braet and colleagues (2008) also collected maternal reports on the children's eating behaviour using the same scale, as well as actual intake via a dietary interview – overweight children were in fact rated by their mothers as demonstrating significantly higher external eating, and this was supported by higher actual energy intake as well. This suggests that self-report measures of intake may be unreliable in children, perhaps as a result of social desirability issues discussed in relation to EAH.

Two studies using the parent-report version of the DEBQ to assess weight differences between clinical groups of overweight children and lean controls have found associations in the expected direction – overweight children have higher scores on 'external eating' (Braet & van Strien, 1997; Jahnke & Warschburger, 2008), although data from an Italian community sample of children aged 11-14 years (n=312) showed no association (Caccialanza et al., 2004).

However, numerous studies have now published data using the two CEBQ subscales designed to measure externality in children ('food responsiveness' and 'enjoyment of food'). Cross-sectional positive associations have consistently been demonstrated with adiposity in ten out of eleven samples identified (all except for Powers et al., 2006), using a number of different samples of children with ages ranging between 3 and 13 years

(Webber et al., 2009; Viana et al., 2008; Spence et al., 2010; Sleddens et al., 2008; Parkinson et al., 2010; Joyce & Zimmer-Gembeck, 2009; Jahnke & Warschburger, 2008; Gregory et al., 2010; Cunha et al., 2010; Carnell & Wardle, 2008a). When researchers have attempted to assess the nature of the relationship over the whole spectrum of weight, results show a linear association between these eating behaviours and adiposity (Webber et al., 2009; Viana et al., 2008; Spence et al., 2010; Parkinson et al., 2010; Cunha et al., 2010; Carnell & Wardle, 2008a), suggesting that these traits do not simply distinguish the clinical from the non-clinical but are systematically related to adiposity across the continuum.

Other studies that have used unstandardised parent-report measures of food responsiveness (e.g. 'overeating' tendency, 'demandingness' regarding food, 'bottle-emptying', or general appetite avidity) with very young children or infants have also found associations in the same direction (Li et al., 2008; Engle & Zeitlin, 1996; Parkinson et al., 2010; Dubois et al., 2007a). Of particular interest is a study by Li and colleagues (2008) who showed that infants who frequently emptied their bottle of milk of their own accord during the first 6 months of infancy were more likely to have excess weight in the second 6 months of life (n= 1896). Also of interest is Wright and colleague's (2006) finding that a better general appetite measured using a single item ('At present, how is your baby/child's appetite' with five response options 'very poor', 'poor', 'all right', 'good', 'very good') in a population-based sample of British infants (n=749) at 6 weeks of age predicted weight gain between 0 and 12 months, and a poorer appetite predicted sustained weight faltering over the same period. This study suggests that appetite quality in infancy is a key driver of growth over the first few months of life, and implicates an up-regulated appetite in the process of rapid growth. A follow-up paper in the same sample a few years later found that a better general appetite (measured in the same way) at 5-6 years predicted higher BMI at 7-8 years, although the infancy appetite ratings at 6 weeks and 12 months were not significantly associated with BMI at this much older age, although the sample size was much reduced (n=344) (Parkinson et al., 2010).

Together these parent-report psychometric studies provide strong evidence in support of the behavioural data that a greater responsiveness to food (or milk) is a characteristic that distinguishes overweight children and infants from their normal-weight peers. In addition, there is some longitudinal evidence which points to the role that this trait may play in the

development of excessive weight during infancy and childhood implicating a causal role for heightened responsiveness to food – i.e. behaviours indicate of food responsiveness appear to be antecedents rather than consequences of overweight.

1.4.3. Sensitivity to internal cues of satiety[4]

1.4.3.1. Microstructural analysis of ingestive patterns

Cross-sectional studies of eating rate have shown fairly consistently that heavier children tend to eat faster than lighter children. On the whole, early studies that assessed eating speed during a standardised meal in naturalistic settings (school cafeterias or at the children's homes) found differences between groups of overweight and normal-weight children ranging from 1-12 years of age (*n*=60; *n*=8; *n*=20) (Drabman et al., 1979; Waxman & Stunkard, 1980; Keane et al., 1981), although one study with 7-12 year olds failed to find a difference (*n*=60), perhaps because the children were not given a standardised meal (Israel et al., 1985).

Later laboratory-based studies have tended to support the positive findings of earlier naturalistic studies. Barkeling and colleagues (1992) showed that 11-year old overweight children (*n*=43) consumed more grams per second of Swedish hash than the normal-weight controls, measured using a universal eating monitor. In a more recent but very similar laboratory-based study, Lindgren and colleagues (2000) again compared the eating speed of Swedish hash in obese and normal-weight children who ranged from 5-18 years (*n*=40), using a universal eating monitor; they found a trend for faster eating in the obese group compared with normal-weight controls (42 grams/min versus 28 grams/min), although this did not quite reach significance (p=0.07) given the small sample size. A third laboratory-based study tested the eating rate of yoghurt in 8-12 year old children of different weight groups (*n*=80), either in the presence of their mother or alone (Laessle et al., 2001); overweight children demonstrated a faster eating rate (grams/second) than the leaner participants when their mother was in the room, perhaps because the presence of the mother resembled the usual eating situation at home, in contrast to the unfamiliar

[4] See Appendix 1.2 for a summary table of the literature.

laboratory situation when the child sat and ate alone. The different food type of yoghurt rather than a meal may also have altered the eating behaviour of the children.

In order to explore eating speed in a more naturalistic setting we recently measured this behaviour in 8-11 year old twins (*n*=254) while they ate a standard lunch of sandwiches and fruit in their own home. The children's behaviour was videotaped so that the number of bites taken per minute could be coded later. In addition to normal-weight/ overweight differences, we explored gradation of effect across lower- and higher-normal-weight. A linear association was observed between eating speed and adiposity across the whole weight continuum showing that rapid eating, like food responsiveness, is not simply a characteristic that distinguishes the obese from the non-obese, but appears to relate to weight linearly (Llewellyn et al., 2008).

A very recent longitudinal study with young children explored if speed of eating at age 4 could distinguish children at lower or higher obesity risk (indexed using parental weight status), and predict greater weight gain between 4 and 6 years (*n*=61) (Berkowitz et al., 2010). The researchers used a concealed videotape to observe and later code the eating behaviour of the children during a standardised laboratory meal in which they were allowed to interact in the usual manner with either their mother or their father. This more naturalistic style laboratory-based study showed that taking a higher number of mouthfuls of food per minute during the meal was an eating style that distinguished the children at higher risk of later obesity from those at lower risk. In addition, a more rapid eating style significantly predicted positive BMI change over two years, as well as increased skinfold thickness, fat, and body fat percentage, and faster eating along with a shorter overall meal duration predicted overweight or obese weight status at age 6. This study suggests that, like 'food responsiveness' eating speed may play a causal role in weight gain in early life.

Two earlier laboratory-based longitudinal studies with infants used sucking rate for milk as a marker of feeding speed, and the findings also provided support for a potential causal role for rapid feeding and weight gain in very early life. More rapid sucking at 2 and 4 weeks of age significantly predicted greater adiposity at 1 and 2 years of age (*n*=99) (Agras et al., 1987), although significance was not maintained as late as 3, 6 or 9 years perhaps as a result of the much reduced sample size due to attrition (*n*=54) and consequently, decreased power (correlations were 0.35, 0.29, 0.33 and 0.21 for 1, 2, 3

and 6 years) (Agras et al., 1990; Agras, 2004). Sucking speed also differentiated infants at higher or lower risk of obesity, based upon parental weight status (*n*=78), and vigorous sucking behaviour at 3 months of age predicted adiposity at 1 and 2 years of age (Stunkard et al., 2004), suggesting a causal role for this behaviour.

An early intervention study succeeded in slowing eating speed in children (by encouraging them to put their utensils down between bites of food) and this resulted in them consuming less food over a 6-month period, although no weight loss was reported, perhaps unsurprisingly considering the small effect size of eating speed on amount consumed, and the relatively short period of time (Epstein et al., 1976). This finding, however, points towards the possibility that this trait can be modified successfully and reduce food intake.

Few studies have assessed whether overweight children exhibit less deceleration in their eating rate over the course of a meal than do their normal-weight counterparts. The laboratory-based studies described earlier by Barkeling and colleagues (1992) and Lindgren and colleagues (2000) found that as well as eating more quickly, the overweight and obese children did not decelerate their eating rate towards the end of the meal to the extent demonstrated by the normal-weight children. Similarly, Laessle et al (2001) showed that obese children demonstrated non-decelerated eating patterns (accelerating their rate towards the end of the meal), as well as a faster eating speed, when their mother was present. In our recent study, although we observed an association between eating rate and weight we did not find that greater adiposity was associated with greater deceleration over the course of the meal (Llewellyn et al., 2008). However, due to the naturalistic setting with less measurement accuracy of intake (as opposed to a universal eating monitor) we used a slightly different method of analysis to characterise deceleration by dividing the mealtime into quarters rather than plotting a cumulative intake curve, which may help to explain the different findings.

Sophisticated experimental studies with adults in which a number of different variables have been manipulated have indicated that both eating rate and deceleration over the course of a meal are influenced by a wealth of factors including food deprivation, food palatability, age, sex, and even portion size (Spiegel, 2000). The many studies described here have used foods with different properties in variable quantities making it difficult to compare the findings meaningfully. Further research in to the microstructure of eating in

children and infants is needed to shed light on when and why faster eating rates and non-decelerated eating patterns emerge. Notwithstanding sample heterogeneity, it is clear that a wealth of evidence supports the hypothesis that greater adiposity is associated with a more rapid eating style, both in laboratory-based and naturalistic settings. It also seems that there is a tendency for overweight and obese children to sometimes lack the 'normal' pattern of satiation over the course of the meal. Moreover, fairly convincing longitudinal evidence from studies with young children and infants suggests that a more avid feeding or eating style may play a causal role in excessive weight gain early in life. While eating speed and deceleration have been thought of as behavioural measures of internal satiety, it is noteworthy that eating speed and eating deceleration may also, in part, tap the same underlying trait that is captured by 'food reinforcement' and 'habituation' paradigms – i.e. the number of responses that a child is willing to make to obtain food indicative of motivation (similar perhaps to eating or sucking speed), and the speed with which the motivation around the food wanes over time (similar perhaps to eating deceleration).

1.4.3.2. Preload compensation

Studies that have assessed differences in satiety sensitivity using a preload paradigm have reported inconsistent results. Johnson & Birch (1994) were the first to assess weight-related differences in compensation accuracy which they demonstrated in 3-5 year old children (n=77). On two separate occasions children were given cherry flavoured drinks matched for volume, mass and sensory properties but differing in energy content (150 kcal versus 3 kcal); 20 minutes later their ad libitum energy intake from a standardised mixed buffet was measured. Poorer compensation after the high-energy drink was modestly associated with greater adiposity in girls but not boys. When this study was replicated in a school setting with 3-5 year-old children (n=77-95) and enhanced by providing the children with familiar preloads – the low-energy drink was water, and strawberry milkshake was used for the high-energy drink – poorer compensation ability was significantly associated with adiposity in boys and girls (unpublished data – S Carnell, EL Gibson and J Wardle), suggesting that overweight children may be less likely to compensate for familiar foods. In keeping with these, another case control study found that 8-12 year old obese children did not down-regulate intake of a range of palatable snacks approximately 10 minutes after a 146 kcal preload of the same palatable snacks (high in fat and sugar), compared with a no preload condition, while the normal-weight children

compensated, suggesting greater satiety sensitivity in the normal-weight children (*n*=31) (Jansen et al., 2003).

However, three other studies failed to find an association. Faith et al (2004) used Johnson and Birch's (1994) study protocol with a 25-minute interval between preloads and subsequent food intake in a sample of 3-7 year olds (*n*=32), but no association with adiposity was found; rather, all children demonstrated reasonable compensation accuracy, although the sample was small making it unlikely that a significant association would be detected. Cecil et al (2005) used three differing preload conditions with a no-energy condition (250 ml water), and low- and high-energy conditions which both used a 250 ml orange drink and a 56 gram muffin matched for taste and mass but differing in energy content (187 kcal versus 389 kcal) to assess weight-related differences in 6-9 year old children (*n*=74). Ninety minutes later the children were given a test meal on an individual tray and intake was measured. No association was observed between intake and weight, and, likewise, most children adjusted their intake to some degree but with the younger children showing better compensation ability than the older ones. In a more recent study, Johnson and Taylor-Holloway (2006) investigated whether the precision of food intake regulation was related to weight in 5-11 year old children (*n*=262) whose intake was measured on two occasions following juice preload drinks of different calorie contents (150 kcal versus 3 kcal). They also failed to find an association with weight, finding instead that most children showed incomplete adjustment to their intake, and in keeping with the findings of Cecil at al (2005), that precision declined further with age.

Lastly, one study has explored this phenomenon with 1-2 year old children who 'fail to thrive', to see if the clinical group of underweight children over-compensate for the preload in the following meal compared with the normal-weight control group (n=*53*) (Kasese-Hara et al., 2002). On two separate occasions the children were given a 150 ml fruit-flavoured drink that was either low in energy (0 kcal) or high in energy (96 kcal) 25 minutes before an ad libitum meal. In both conditions, the underweight children consumed less at the meal than the normal-weight children, and the underweight children also consumed significantly less of the preload, but they failed to compensate for the preload in the same way as the normal-weight children, instead eating more following the high energy drink than the low energy drink. This may indicate some disruption to internal satiety signals in seriously underweight children.

Methodological heterogeneity across these studies may go part way towards explaining the different findings. Cecil et al (2005) had solid food as well as liquid in their different preloads as well as a considerably longer interval (90 minutes) than those studies that reported significant findings (10-20 minutes) which may have improved compensation across the board. Faith et al (2004) who replicated Johnson and Birch's study had a sample less than half the size, and the authors themselves suggested that the study was underpowered to detect a small effect which may explain the null finding; larger sample sizes may be needed to reliably detect the typically weak association between compensation ability and weight. The majority of the samples in the studies reporting associations were White Caucasian, while just over half of Johnson and Taylor-Holloway's (2006) sample was Hispanic, and the majority of Faith et al's (2004) was of African-American or Hispanic origin; it is not inconceivable that ethnicity plays a role in influencing this behavioural trait given that eating behaviour is a highly context-dependent phenomenon for which cultural norms play a central role. Lastly, children who have been referred to secondary care because they are failing to thrive may have a host of underlying problems (psychological and organic) that are unrelated to appetite, thereby not truly representing the lower end of the weight distribution but potentially representing disordered eating behaviour.

1.4.3.3. Psychometric measures of eating speed and internal cues of satiety

Psychometric studies have provided much more consistent evidence that a higher speed of eating is related to greater adiposity, cross-sectionally. All of the studies that have used the 'slowness in eating' subscale of the CEBQ have found a significant association with adiposity in the hypothesised direction across a range of samples and ages (Carnell & Wardle, 2008a; Cunha et al., 2010; Parkinson et al., 2010; Sleddens et al., 2008; Spence et al., 2010; Viana et al., 2008; Webber et al., 2009), and show that 'slowness in eating' decreases linearly with weight in a graded fashion across the spectrum when the sample has been divided into weight groups (Carnell & Wardle, 2008a; Cunha et al., 2010; Parkinson et al., 2010; Spence et al., 2010; Viana et al., 2008; Webber et al., 2009). Studies that have explored this trait using unstandardised questionnaire measures have also reported significant findings (Sugimori et al., 2004; Jahnke & Warschburger, 2008; He et al., 2000), and Sugimori and colleagues (2004) used a prospective design to show that

faster eating at 3 years of age predicted change from normal-weight status to obese status at age 6, and predicted maintenance of obesity from age 3 to 6 in a very large sample of Japanese children (*n*=7693).

A wealth of psychometric studies have also used the CEBQ to associate the 'satiety responsiveness' subscale with adiposity, and all have found a negative association across different ages (Webber et al., 2009; Viana et al., 2008; Spence et al., 2010; Sleddens et al., 2008; Parkinson et al., 2010; Cunha et al., 2010; Carnell & Wardle, 2008a); in each case, satiety responsiveness appeared to decrease linearly as weight increased (Webber et al., 2009; Viana et al., 2008; Spence et al., 2010; Parkinson et al., 2010; Cunha et al., 2010; Carnell & Wardle, 2008a). Parkinson (2010) entered all of the CEBQ scales into an analysis simultaneously to identify which of the CEBQ traits measured at 5-6 years were independently associated with BMI at 7-8 years and 'satiety responsiveness' was identified as a significant predictor of weight longitudinally.

Collectively, these psychometric studies support the behavioural studies indicating that in general, as eating speed increases, so does weight, and this trait may play a role in the development and maintenance of excess weight. Moreover, a child's sensitivity to his or her internal cues of satiety also appears to be of importance in distinguishing children with greater adiposity from those with lower adiposity, and this may be a key trait related to weight gain in childhood.

1.4.4. Food preferences[5]

A number of studies have investigated the relationship between food preferences and adiposity in children to identify if increased liking of 'unhealthy' foods or less liking for 'healthy' foods can predict overweight, but findings are not consistent. Fisher and Birch (1995) found that fat preferences in 3-5 year old children (*n*=18) were related to triceps skinfold measurements, although not to subscapular skinfold; Ricketts (1997) also found that fat preferences were related to triceps skinfold but not to subscapular skinfold, and they were also related to BMI in 9-12 year olds (*n*=88); both studies found fat preferences to be positively associated with actual fat intake. In a very large sample of Chinese

[5] See Appendix 1.3 for a summary table of the literature.

children ranging from 6-19 years (*n*=5755) higher preference for vegetables appeared to offer a protective factor, being associated with lower odds for overweight (Xiong et al., 2008), and this finding was supported in a US sample of 10-11 year olds (*n*=341) (Lakkakula et al., 2008).

On the other hand, a small study with adolescents and young adults aged 12-22 years (*n*=26) found no difference in fat preferences between the obese and normal-weight, although the study would have been underpowered to detect small effects (Fieldstone et al., 1997). A much larger study (*n*=366) conducted more recently also found no associations between adiposity and preferences for fatty and sugary foods, nor for fruits and vegetables in 7-9 year old children (Hill et al., 2009b).

Using parental BMI as an index of obesity risk in 4-5 year olds twins (*n*=428), Wardle and colleagues (2001a) found that children at higher-risk had greater preference for higher fat foods (assessed behaviourally), and lower liking for various vegetables (as reported by the mother), implicating these preferences in the aetiology of predicted future weight. This was also supported in the study by Fisher and Birch (1995) who found that parental BMI was associated with child fat preference, as well as the child's own adiposity measures.

The inconsistency of these findings may reflect the distinction between 'liking' and 'wanting', which have been found to be neurologically distinct appetitive traits (Berridge, 1996; Berridge, 2004). While it is primarily liking (or reports of liking) that are tapped in preference questionnaires, it may be wanting that drives food choices. Wanting a food might be indexed more effectively by behavioural reinforcement paradigms (e.g. Lappalainen & Epstein, 1990), in which a child is forced to choose between alternative foods. In support of this, Saelens and Epstein (1996) found that while both obese and non-obese women gave equivalent liking ratings to food and non-food rewards, the food reward had a much greater reinforcing value for the obese women compared to the non-obese women in behavioural tasks. Additionally, in studies investigating the reinforcing value of food, there was a significant correlation between reinforcing value and energy intake, but no relation between food liking and intake, and the reinforcing value of food was not correlated with food liking (Temple et al., 2008). This highlights the importance of distinguishing between the desire or drive to eat certain foods and the actual pleasure gained from eating them.

Recent research has also indicated that additional factors such as unconscious psychological processes may moderate the relationship between adiposity and food preferences. Halford and colleagues (2007a) observed an interaction between weight status and food branding in the prediction of food preferences such that overweight children preferred more branded than unbranded high fat foods, while normal-weight children preferred more unbranded than branded carbohydrate foods. On the whole, overweight and obese children preferred more branded foods than the normal-weight controls suggesting that overweight children may be more susceptible to the advertising of high fat foods which could in part be driving their liking for them. More research is needed in this area to uncover the implicit affective and motivational aspects of food preferences.

1.4.5. Food fussiness[6]

Excessive fussiness with regard to food, or 'picky eating', has primarily been investigated by comparing 'picky' with 'non-picky' eaters (Harris, 1993), and has been linked with lower weight, lower rates of overweight, and failure to thrive (Galloway et al., 2005; Dubois et al., 2007a; Carruth et al., 2004; Ekstein et al., 2010), although these findings have not always been replicated (Rydell et al., 1995; Carruth et al., 1998; Wright et al., 2007). Recently, a number of studies have examined the nature of the relationship between fussiness and weight in non-clinical samples using the CEBQ, but results have been equally inconsistent.

Webber and colleagues (2009) found a significant negative association between food fussiness and weight in two samples of British children aged between 7 and 12 years (n=239; n=167) showing that thinner children tend to be fussier around food, and this finding was replicated in a number of other samples of Portuguese and Canadian children ranging from 3-13 years (Viana et al., 2008; Spence et al., 2010; Cunha et al., 2010) suggesting that fussiness could be protective against overeating, perhaps by reducing the number of foods a child is willing to eat. The same effect was not replicated in a recent Dutch study using the same measure in 6-7 year olds (n=135), although the pattern of results indicated a trend in the same direction (Sleddens et al., 2008), nor was fussiness

[6] See Appendix 1.3 for a summary table of the literature.

related to adiposity in an Australian sample of much younger children aged 2-4 (*n*=156) (Gregory et al., 2010).

Despite some cross-sectional evidence linking fussiness with underweight in children, research showing how fussiness relates to weight over the longer-term is lacking. Also some research with adults has observed greater fussiness around food in obese people to the extent that efforts to explore group differences in eating patterns using microstructural analysis in the laboratory have been hampered because of the difficulty of finding a test food that enough of the obese participants found acceptable (Guss & Kissileff, 2000). Data from early animal studies have supported this phenomenon – for example, obesity-inducing lesions in the ventromedial hypothalamus in rats produced 'finicky' behaviour insofar as the obese rats were unwilling to consume the bitter-tasting food, but would overeat on the highly palatable food (Schachter, 1971). On this basis we might expect to find that the association between fussiness and weight depends on the quality and palatability of the food supply. Perhaps fussy eaters in the 1950s were unlikely to become overweight, but in the current food environment they can. Longitudinal studies that observe the weight trajectories of childhood fussy eaters into adulthood will help to clarify how this trait relates to weight over the long-term.

1.4.6. Emotional eating[7]

Studies relating emotional eating to weight in children have also had mixed results, and the findings appear to be influenced by the reporter and the sample. The 'emotional eating' subscale of the DEBQ which measures the tendency to overeat in response to negative emotions has shown all possible relationships with weight. Only one study found that obese children aged 9-12 years scored significantly higher than non-obese children (*n*=292), but this study utilised a clinical sample (Braet & van Strien, 1997). Another study using a clinical sample and six studies that utilised non-clinical samples found no association between emotional eating and weight in children ranging from 3-18 years of age in various populations including Dutch, Belgian, Italian, German, American and British (Wardle et al., 1992; van Strien & Oosterveld, 2008; Jahnke & Warschburger, 2008; Caccialanza et al., 2004; Braet et al., 2008; Jollie-Trottier et al., 2009). To 'muddy the

[7] See Appendix 1.4 for a summary table of the literature.

water' further, two studies have reported a negative association between emotional eating and weight – Hill and colleagues (1994) found underweight girls to have the highest score on emotional eating, with the overweight girls scoring lower, in a community sample of 9 year old children (*n*=379), while a negative association has been reported among a large sample of 9-10 year old children (*n*=2379) using a bespoke psychometric measure of emotion-induced eating (Striegel-Moore et al., 1999). However, both of these studies used child self-report measures which may render the findings unreliable.

A number of studies using the CEBQ have assessed the relationship between both emotional overeating and emotional under-eating (EOE, EUE). A number of studies have supported the hypothesis that higher rates of eating in response to negative emotions are observed in overweight children than normal-weight children (Webber et al., 2009; Viana et al., 2008; Spence et al., 2010; Joyce & Zimmer-Gembeck, 2009; Cunha et al., 2010), and Parkinson and colleagues (2010) found that this trait at age 5-6 years was associated prospectively with BMI at 7-8 years, independently of the other CEBQ traits. But these findings have not always been replicated – a recent study with a community sample of 6-7 year old children found no association between 'emotional overeating' and weight (*n*=135) (Sleddens et al., 2008).

The picture is less clear still with 'emotional under-eating' and weight. Viana et al (2008) found a significant but weak negative association between EUE and weight showing that thinner children tend to undereat more often than fatter children in response to negative emotions, and a very small but significant association was also reported by Cuhna and colleagues (2010). However, just as many other studies have not found a significant association (Webber et al., 2009; Spence et al., 2010; Sleddens et al., 2008). It is also noteworthy that studies that found significant positive associations between 'emotional overeating' and weight did not always find significant negative associations with 'emotional under-eating' (e.g. Webber et al., 2009; Spence et al., 2010) indicating that overeating and under-eating in response to emotional distress are not opposite ends of the same dimension, nor are they mutually exclusive (Webber et al., 2009).

Methodological heterogeneity may account for some of the different findings (different age groups, ethnicities and measures), but an alternative explanation is that the association can vary. Stress research with animals has indicated that both over- and under-eating

occurs in response to stress. A recent study with university students found that both gaining and losing weight were associated with more stress than weight stability, suggesting that individuals may vary in their response to stress, with hypophagic and hyperphagic eating behaviours being possible, depending on the nature and intensity of the stressor and subsequent stress response (Serlachius et al., 2007).

1.4.7. Behaviours observed in 'failure to thrive'[8]

A handful of studies have observed behaviour during meals (or asked parents to report on their children's mealtime behaviour) of clinical samples of children who are 'failing to thrive' and compared it to children without problems in an attempt to identify the problematic behaviour causing the reduced food intake. Some of the observations, although not all, indicate that these children demonstrate the other extreme end of the characteristics that distinguish overweight children from their normal-weight counterparts – that is they show indications of an extremely down-regulated appetite. For example, Wright and colleagues (2000) reported on two samples of 15-18 month old British children with 'failure to thrive' and compared their mealtime behaviour with that of normal-weight controls (n=125; n=89). In one sample the severely underweight children were more often rated by their mother as a 'variable eater' compared with the normal-weight children who were more often described as 'hungry'; the clinical group also differed in that they did not like most foods, i.e. were fussier. Similarly, in the other sample failing to thrive children were less 'hungry' at mealtimes, and ate fewer foods. In keeping with these findings Wilensky et al (1996) used a structured interview with a very large sample of parents (n=1407) of 25 month old infants to identify problematic feeding behaviours and found that children who were 'failing to thrive' showed significantly less hunger, ate a significantly smaller variety of foods, and showed significantly less enjoyment during mealtimes compared to children without problems.

During meals, severely underweight children consume significantly less energy than the normal-weight children (Parkinson et al., 2004; Drewett et al., 2003). An observational study of a group of Mexican children with growth-faltering aged 2-5 years (n=45) allowed

[8] See Appendix 1.5 for a summary table of the literature.

the children to roam freely within the experimental setting and take and consume food as they wished throughout the course of a day (Garcia et al., 1990). These children consumed considerably fewer calories than were available to them, highlighting that the reduced intake observed in other studies may be determined by the child rather than the parent or other extraneous factors, opening up the possibility that these children have extremely down-regulated appetites. This is supported by Wright and colleague's (2006) finding (described earlier) that a poorer appetite predicted sustained weight faltering from 0-12 months.

1.5. Conclusions and future research

This review has evaluated the research literature on the relationships between adiposity and distinctive appetitive traits in children and infants. There is convincing evidence that behavioural indicators of food responsiveness and sensitivity to internal cues of satiety are cross-sectionally associated with weight in children and infants. Not only this, researchers have also made the case that these characteristics may play a causal role in the accumulation of excessive weight gained during the developmental years by providing evidence of prospective associations. Nevertheless, associations have not been consistently found, especially in behavioural studies. Another observation is that very little work has been done to explore whether the same types of appetitive traits manifest themselves in infancy, and if so how they relate to weight. This is of considerable interest given the importance of early life weight gain for later obesity risk. The lack of research in the early life period has in part been hampered by the absence of a standardised psychometric measure to allow for large-scale research, such as has been permitted with the CEBQ. These points are discussed in more detail below.

1.5.1. Limitations of behavioural measures of eating behaviours

Possible explanations for the varied findings from the behavioural studies (e.g. 'eating in the absence of hunger', microstructural analysis of eating and preload compensation) include heterogeneity across studies of factors that appear to be related to the expression of these traits. One such factor may be the properties of the food used such as palatability,

form (solid or liquid), texture, macronutrient content and even portion size. Another possible influence is the level of social exposure inherent in the testing situation, which may encourage social desirability to play a role, especially among girls. The characteristics of the sample may also be important, including the age and sex of the children: some studies have suggested that these traits show less variability in younger versus older children; sex differences have also been reported, although this may sometimes be related to other factors such as social desirability, but other explanations are possible too such as sex differences in the physiology behind appetite regulation. Lastly, the sample size is important because it determines the power of the study to detect significant effects – the relationship between these traits and weight appears to be fairly small so very large samples are required to find associations reliably, which limits the scope of behavioural measures of eating behaviours in this area of research, especially in younger children in whom there may be less variation in these traits.

Psychometric measures of these traits, on the other hand, have shown remarkable consistency across studies when related to weight. This may be due to the increased power of this mode of measurement to find smaller associations by enabling the reporter to average instances of the behaviour over numerous occasions and situations. It could, however, result from parents retrospectively attributing these traits to their children in response to their body size. However, the fact that these traits share a linear relationship with adiposity across the whole continuum makes this unlikely as parents would be required to judge the appropriate frequency of the behaviour in relation to their child's weight with remarkable skill and precision.

1.5.2. Infancy is a critical period with a gap in the research-base

Relatively little research of any sort has been carried out in the area of infant appetite or feeding behaviour. The few studies in this area have been conducted by a limited number of researchers and only one or two feeding styles have been studied. The focus of infant studies has also tended to be infants who are 'failing to thrive' or the description of feeding problems, rather than the characterisation of a normal pattern of feeding that is able to distinguish infants with up-regulated as well as down-regulated appetites. This is an important gap in the literature given that current research is indicating that weight predisposition is expressed by a very young age – early postnatal growth is highly

predictive of later childhood and even adult weight (Lake et al., 1997; Trudeau et al., 2003) – making it important to intervene early. This suggests that causal processes begin soon after birth and assessment at later ages may be less informative should weight predisposition already have been expressed; if eating styles are truly determinative of the development of adiposity it is necessary to assess them in the earliest period of life, so that interventions to try to attenuate the expression of these traits may be developed before excess weight gain has occurred.

There is reason to believe that there are individual differences in appetitive traits from the beginning of life. Kron et al (1968) observed considerable variability in infants' behavioural indicators of appetite avidity that persisted over 18 feeds, showing remarkable intrapersonal stability in these traits at less than 1 week of age. To add weight to this, studies that have related the quantity of milk produced by the mother to the amount consumed by the infant have found that the quantity consumed is primarily determined by the infant – that is, the amount of milk produced by the mother appears to reflect first and foremost the infant's appetite, rather than the other way around (Dewey & Lonnerdal, 1986; Dewey et al., 1991; Drewett & Woolridge, 1981). This research base points towards the possibility that individual differences in appetite from the beginning of life play a role in the development of adiposity postnatally.

1.5.3. Need for a standardised psychometric measure of appetite in infancy

At present no comprehensive standardised measure of infant feeding behaviours or appetite exists. A small number of studies have suggested that individual differences in feeding behaviours indicative of later 'obesogenic' eating behaviours may be present in the first weeks of life, and longitudinal findings implicate a causal role for these feeding characteristics in the accumulation of excess adiposity (Agras et al., 1987; Li et al., 2008; Stunkard et al., 2004). However, there is a need to characterise the full range of feeding behaviours that may favour excessive intake and faster growth, and to conduct well-designed large population-based studies to explore how they relate to weight in early life, and weight gain over development. A validated, reliable and comprehensive psychometric measure of appetite would offer a cheap and convenient method of large scale prospective research into the relationship between early feeding behaviours and weight, early feeding

behaviours and later eating styles, and allow researchers to understand the origins of these traits more fully.

CHAPTER 2. THE ORIGINS OF EATING BEHAVIOURS: GENETIC AND ENVIRONMENTAL INFLUENCES[9]

2.1. Background

If appetitive traits play a causal role in the development of excessive weight gain, as suggested by Chapter 1, it is important to understand the underlying pathways that these traits reflect, where they originate from, and, most importantly, why there are individual differences in these characteristics. Another question that has been of interest is whether these appetitive proclivities are different manifestations of the same underlying causal pathway, or they arise from distinct underpinnings that are independent. A better understanding of the origins of these traits will allow researchers to design early interventions that are effective in attenuating their expression and preventing overweight from developing.

Recent collaborations between researchers in the fields of endocrinology, neurology, and behavioural science have started to uncover the physiological and neurological architecture underlying these appetitive traits. At the most basic level, individual differences in physiological, neurological or psychological aspects of appetite may reflect individual differences in genes. Understanding how genes shape the expression of these traits will allow researchers to build theories about how appetite is influenced from the 'bottom up', starting with the foundations of appetite regulation. Behaviour genetic research is making some progress in determining the relative importance of genes and environment in the shaping of these characteristics, but very little work has been done during the early life period.

This chapter will start by providing a brief overview of what is currently known about the physiological regulation of appetite. The idea that inherited individual differences in appetite may cause individual differences in weight will then be discussed, followed by an overview of current behaviour genetic research into appetite.

[9] Much of this chapter has now been published in a book chapter: Llewellyn C, Carnell S, Wardle J. (2011). Eating Behaviour and Weight in Children. In (Eds) L Moreno, I Pigeot, W Ahrens. Epidemiology of Obesity in Children and Adolescents (Book I of II) - *Prevalence and Aetiology*. Springer series; New York.

2.2. The physiological regulation of appetite

Recent advances in endocrinological and neurological research have furthered existing knowledge about the central and peripheral regulation of appetite by the complex integration of systems within the brain. Eating behaviour reflects both 'homeostatic' and 'hedonic' processes (Blundell, 2006). Homeostatic mechanisms are involved in maintaining energy balance; there are a complex array of processes that manage both short-term fluctuations in energy stores (so-called 'episodic' regulation), and more stable energy reserves (so-called 'tonic' regulation) by integrating signals from the periphery within the brain (Halford & Blundell, 2000). Hedonic mechanisms that reflect and express the sensory pleasure and reward experienced from food (Zheng & Berthoud, 2008) are also involved. These two systems (and their potential interplay) are described in more detail below.

2.2.1. Homeostatic control of appetite

The homeostatic regulation of appetite has been the subject of a number of in-depth reviews (Arora & Anubhuti, 2006; Druce & Bloom, 2006; Murphy & Bloom, 2006; Schwartz et al., 2000; Woods & D'Alessio, 2008; Wynne et al., 2005), and much of the research in this area has been based upon experimental work using animals. This section will provide a brief overview of the key processes thought to be involved. All of the information presented here has been taken from these reviews unless cited otherwise.

The hypothalamus and brain stem coordinate peripheral homeostatic responses to energy availability which enables appetite to be regulated centrally. In particular, within the arcuate nucleus (ARC) of the hypothalamus, two distinct populations of neurons work in concert to modulate feeding behaviour. One set are orexigenic neurons that co-express neuropeptide Y [NPY] and agouti-related peptide [AgRP], and are responsible for upregulating appetite or hunger; the other set are anorexigenic neurons that release proopiomelanocortin [POMC] and cocaine-and amphetamine-regulated transcript [CART]), and act to downregulate appetite or promote satiety.

Both of these sets of neurons extend into the paraventricular nucleus (PVN) and other nuclei involved in appetite control or feeding (e.g. the dorsomedial hypothalamus and the lateral hypothalamic nucleus, LHA), and stimulate secondary neurons involved in energy balance. POMC is the precursor for α-melanocyte-stimulating hormone (αMSH) which stimulates melanocortin 3 and 4 receptors (MC3R and MC4R) on neurons in the PVN to reduce hunger; POMC neurons also innervate neurons in the LHA that release melanin concentrating hormone and those producing orexins (both potent stimulators of hunger), but the net effect of POMC action is to reduce hunger suggesting that it inhibits these cells. AgRP and NPY innervate many of the same areas but with opposing action; AgRP is an antagonist at MC3R and MC4R so counters the activity of POMC and increases hunger, while NPY stimulates Y receptors (predominantly Y1 and Y5) also in the paraventricular nucleus, to increase hunger. The ARC can be accessed by chemical signals carried in the blood (such as circulating hormones) because it is not protected by the blood-brain barrier, and this brain region is sensitive to a large number of energy signals from the periphery that can act centrally to modulate appetite potency.

2.2.1.1. 'Episodic' homeostatic control of appetite

Temporary fluctuations in energy in the context of meal-to-meal variability are modulated primarily by the gastro-intestinal tract. Chemical signals in the form of gut hormones are released periodically in response to gut nutrient content (so-called 'satiety hormones'), or to an absence of food (so-called 'hunger hormones'), and send signals to the brain via sensory nerves stimulated by mechanical receptors in the gut, directly via the vagus nerve, or through the circulatory system. Grehlin, produced predominantly by the stomach, has been identified as a potent 'hunger' hormone and the only known peripheral orexigenic signal whose primary function is to facilitate initiation of eating – exogenous administration of grehlin stimulates food intake (Asakawa et al., 2001; Tschop et al., 2000; Wren et al., 2000; Wren et al., 2001), and levels of grehlin increase in the absence of food and decrease following intake (Ariyasu et al., 2001; Cummings et al., 2002). There are grehlin receptors in the arcuate nucleus of the hypothalamus and the brain stem which are able to integrate signals from this hormone centrally and activate NPY and AgRP neurons to increase appetite when grehlin levels rise.

The so-called satiety hormones or peptides (e.g. peptide YY, glucagon-like peptide, oxyntomodulin, cholecystokinen, and pancreatic polypeptide) are produced by the gut in proportion to the number of calories and the macronutrients consumed. One way that these peptides affect appetite is to influence the rate of gastric emptying, but they also stimulate sensory nerves that connect with the hind brain and trigger impulses in neurons within the ARC to effect satiation (cessation of eating) and post-meal satiety (inhibition of eating following a meal) centrally. For example, peptide YY suppresses the expression of NPY through action on the Y receptors (Abbott et al., 2005), and has been shown to increase expression of POMC (Batterham et al., 2002).

2.2.1.2. 'Tonic' homeostatic control of appetite

As well as adjusting appetite levels in response to temporary oscillations in energy influx from meals, the brain takes into account longer-term energy stores such as adiposity. Important chemical signals include leptin and insulin. Leptin is produced primarily by white adipose tissue in direct proportion to the number and size of fat cells, and insulin is produced by the beta cells of the pancreas also in direct proportion to body fat (as well as in response to food intake). These two hormones circulate in the blood and act on the neurons in the ARC of the hypothalamus to influence appetite in response to the availability of long-term energy stores. Higher leptin levels signal greater energy stores in the body and leptin inhibits the orexigenic neurones (suppressing the release of NPY and AgRP) and stimulates anorexigenic neurons (increasing expression of POMC, alpha-MSH and CART) to decrease overall appetite (Jequier, 2002). Likewise, higher insulin levels reflect greater adiposity, and this chemical signal acts to reduce food intake by inhibiting NPY and increasing POMC (Baskin et al., 1988). Other chemical signals may also be involved such as amylin, visfatin, adiponectin, resistin and certain cytokines (Arora & Anubhuti, 2006; Druce & Bloom, 2006; Murphy & Bloom, 2006; Schwartz et al., 2000; Woods & D'Alessio, 2008).

2.2.1.3. Interaction between tonic and episodic homeostatic mechanisms

Longer-term regulators of energy balance such as leptin and insulin also act to modulate the potency of the 'episodic' signals, allowing multiple energy indicators to be taken into account at any one eating episode. The strength of the 'satiety' signal in the brain from gut hormones is attenuated when energy stores are low (and leptin and insulin are low) so that more energy is consumed during a meal, and conversely it is enhanced if adiposity is high (and leptin and insulin are increased) in order to reduce the amount eaten (e.g. Emond et al., 1999; Figlewicz et al., 1986; Matson et al., 1997; Matson & Ritter, 1999; McMinn et al., 2000; Morton et al., 2005; Riedy et al., 1995).

2.2.2. Hedonic control of appetite

Sensory pleasure and reward systems also influence eating behaviour – i.e. the desire to eat certain foods regardless of the body's homeostatic state – and this probably involves neurologically dissociable processes related to 'learning' (representations of the palatability of the food in memory), 'liking' and 'wanting' (Berridge, 2003). Eating that is driven primarily by liking or wanting of palatable food, rather than nutritional need, is referred to as 'hedonic' appetite, i.e. eating for pleasure (Zheng & Berthoud, 2008). Hedonic control of eating probably involves a number of systems that are separate from the homeostatic mechanisms, including dopamine pathways, the opioid system, and endocannabinoids (Zheng & Berthoud, 2008). In particular, subjective liking of food is believed to involve the mu-opioid (Pecina & Berridge, 2000) and endocannabinoid systems (Mahler et al., 2007), while wanting is believed to be primarily underpinned by the mesolimbic dopamine system (Dayan & Balleine, 2002). A number of recent studies suggest that the sensory properties of food are represented in the orbitofrontal cortex, an area in the prefrontal cortex involved in the integration of information from all sensory modalities including taste, texture, colour, smell and reward value (de Araujo et al., 2005; Verhagen, 2007).

2.2.3. Homeostatic and hedonic interaction

Although homeostatic and hedonic mechanisms are rooted in separate systems, there is reason to believe that there is substantial interplay between the two. Aspects of the homeostatic system can modulate the hedonic control of appetite and vice versa. Leptin and insulin can downregulate wanting through action on dopaminergic neurons in the mesolimbic pathway (Figlewicz, 2003; Fulton et al., 2006; Hommel et al., 2006), while grehlin facilitates reward processing (Abizaid et al., 2006; Jerlhag et al., 2007). On the other hand, reward processes are activated in response to the consumption of palatable food (high in fat and sugar) and can override homeostatic satiety mechanisms in the hypothalamus to prolong eating (Erlanson-Albertsson, 2005).

Biology clearly plays an important role in the regulation of appetite, and differences in genes that influence any of these processes may account for individual differences in appetitive traits such as sensitivity to internal satiety mechanisms (e.g. homeostatic processes) and responsiveness to external food cues (e.g. hedonic processes). If there are genes that influence appetite, these genes may also influence weight through appetitive pathways, giving rise to the observed associations between appetitive traits and weight described in Chapter 1, and contributing, at least in part, to the heritability of weight. This hypothesis is discussed in more detail in the following section.

2.3. A behavioural susceptibility model of weight

2.3.1. The heritability of body weight

Family studies and twin designs have long been used by behavioural researchers to quantify the relative influences of genes and environment on any given trait. The essence of the method is to compare the magnitude of associations between relatives of differing genetic relatedness for a particular trait; if resemblance is greater for more closely related individuals (e.g. siblings versus parents) then genetic influence is inferred, but if associations are the same regardless of genetic similarity, then environmental influences may be assumed (Plomin et al., 2008).

Family studies are a good starting point – if traits aggregate in families, it is an indication that shared genes may be contributing to within-family likeness. But shared genes and shared environments are confounded in families, and covariation could be explained either by important environmental influences that families share in common, or by genes. Twin data offer a powerful alternative design because they allow the relative contribution of genes, shared family environments and the individual's unique environment to be determined. The resemblance between monozygotic (MZ) twins who are genetically identical is compared with that between dizygotic twins (DZ) who share approximately 50% of their segregating genes, but are similar to MZ twins in that they are the same age and grow up at the same time, in the same family (i.e. their shared environments are assumed to be the same). This means that we can calculate the genetic influence on the trait of interest (the 'heritability' estimate) by doubling the difference between the MZ and DZ correlations; twin resemblance not attributable to genes is considered to reflect shared environmental influences and can be estimated by subtracting the heritability estimate from the MZ correlation, while remaining variance is apportioned to unique, non-shared environmental effects and measurement error (Plomin et al., 2008).

In general, family studies are only equipped to produce 'familiality' estimates which indicate the proportion of variance explained by family influences (shared genes and shared environments), although attempts have been made to estimate 'heritability' (an index of the genetic effect size), by including more unusual designs such as full and half siblings living within the same family, and by adjusting for covariation accounted for by living in the same home. Heritability estimates from twin studies are easier to interpret because the genetic and environmental influences are completely partitioned out. The most powerful design is the adoption study that can directly estimate genetic effects (e.g. biological parents and adopted away children) or shared environment effects (e.g. adoptive parents and adopted children), although these are increasingly rare. The methods used to derive heritability estimates from quantitative genetic analyses, and the strengths and weaknesses of different designs, are explained in detail in Chapter 4.

The heritability of body weight has been estimated using family, adoption and twin studies, and has been the subject matter of a number of reviews (Bouchard, 2009; Grilo & Pogue-Geile, 1991; Maes et al., 1997; Silventoinen et al., 2010). Although estimates of heritability

tend to vary with the types of relatives included and the analytic methods applied, genetic influences on weight have been firmly established across numerous samples, and at every stage of the lifespan. Weight tends to be correlated within families – e.g. an adult with a severely obese (BMI ~ 40) first degree relative is about 5 times more likely to become as severely obese compared to individuals with only normal-weight relatives (Lee et al., 1997). The BMIs of adopted children are significantly associated with the body size of their biological parents with whom they only have genes in common, but are not correlated with that of their adopted parents with whom they only share environmental factors (e.g. Sorensen et al., 1989; Stunkard et al., 1986b). Body size is more highly correlated in MZ than DZs pairs (Stunkard et al., 1986a; Turula et al., 1990; Wardle et al., 2008a), and correlations between MZs who have been reared apart are almost as high as those of MZs reared together (Grilo & Pogue-Geile, 1991), suggesting it is shared genes rather than shared environments accounting for the physical resemblance, in keeping with the adoption studies.

Estimates of heritability generated from adoption and family studies tend to be the lowest, between about 20% and 80%, while twin studies typically yield the highest estimates in the region of 50% to 90% (Bouchard et al., 2004a; Bulik et al., 2003; Maes et al., 1997; Malis et al., 2005; Romeis et al., 2004; Schousboe et al., 2003). There are two likely reasons why adoption and family studies find lower heritability estimates than twin studies. Firstly, genetic effects may be age-dependent, insofar as the expression of genes can vary over time, and different genes may be involved at various stages of development (Plomin et al., 2008). In support of this, some family studies have shown that the weight of siblings is more highly correlated in those who are closer in age (e.g.Mueller & Malina, 1980), and twin studies that have estimated the correlation between genetic effects for adiposity across multiple age points have found that it is high, although not unified (e.g. Haworth et al., 2008). Secondly, non-additive genetic effects (interactions between genes such that the effects do not simply add up) are not correlated between parents and off-spring and only correlated slightly in siblings, while they are correlated completely in MZ twins (this is discussed in detail in Chapter 4). The net effect of non-additivity is to lower heritability estimates from family and adoption studies, although it can be tested for and taken into account in twin studies. Some reviews have estimated that up to half of the genetic variance on weight is due to non-additive genetic effects (Maes et al., 1997). A recent review of twin and adoption studies during childhood (1 to 18 years) concluded that

genetic influences on body weight were strong throughout the developmental years, and their meta-analysis produced a heritability estimate of about 70% that took account of genetic influence across the ages (Silventoinen et al., 2010).

Studies that have focused on the infancy period have indicated that genes play a role in weight regulation from very early in life. However, the influence of genes on birth weight appears to be modest, compared to influences a few months after birth. A very early review concluded that only about 10% of the variation in weight at birth was due to genetic factors (Robson, 1978). Although estimates from more recent studies have varied greatly (from ~20% to ~80%, e.g. Demerath et al., 2007; Vlietinck et al., 1989), twin studies tend to be consistent in reporting estimates in the region of 20% to 40% (Dubois et al., 2007b; Vlietinck et al., 1989; Whitfield et al., 2001), and very large (n>100,000) and well-designed family studies with sufficient power to estimate heritability reliably have indicated that it is likely to be in the region of 25% to 31% (Lunde et al., 2007; Magnus et al., 1984). However, genetic influences on weight increase over the first few months of life, and genes appear to influence both weight at given time-points as well as indices of growth. At three months estimates have been reported between 28% and 67% (Demerath et al., 2007; Levine et al., 1987), and from around 5 to 6 months the heritability of weight has increased substantially to 65% to 90% (Demerath et al., 2007; Dubois et al., 2007b; Levine et al., 1987); the heritability of weight change between birth and 6 months has been estimated as 66% (Demerath et al., 2007), while similar estimates have been reported for indices of growth rate between birth and 12 months (52%) (Livshits et al., 2000), and birth and 24 months (57% to 63%) (van Dommelen et al., 2004).

Collectively, this research base points towards the importance of genetic influences on weight regulation from the beginning of life onwards, raising the question of how genes are influencing body weight. The potential role of appetite in mediating these genetic effects is discussed below.

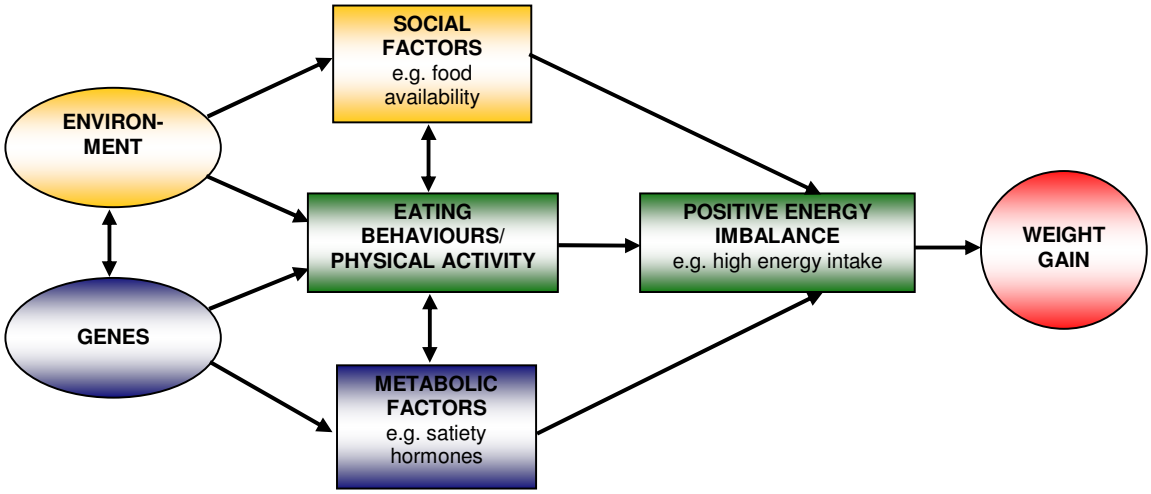
2.3.2. Genetically-determined appetitive traits and the heritability of weight

Attempts to unravel the genetic architecture behind the control of body weight and growth have focused in the main on examining the genetic variation in the physiological processes

that directly affect adiposity, such as fat metabolism and energy expenditure (Froguel & Boutin, 2001; Mutch & Clement, 2006). However, in recent years the spotlight has turned on appetite as an important pathway through which the genetic control of weight may be mediated at least in part. Firstly, obesity is the result of positive energy imbalance resulting from the ingestion of more energy than is expended, making eating behaviour a likely culprit. Recently, it was shown by Swinburn et al. (2009) that the increases in the US population weight over the last 30 years can be almost entirely explained by increases in energy consumption, rather than expenditure. Secondly, the dramatic increase in obesity prevalence over the last 30 years suggests an interaction between obesogenic genotypes and environmental factors, as the gene pool cannot have changed in 3 decades; such an interaction would be possible if inherited individual differences in appetitive traits, including higher food responsiveness and lower satiety sensitivity, bestow increased risk of weight gain for some individuals under certain (current) environmental conditions, such as high availability of palatable food. A behavioural susceptibility model of obesity makes sense of the seeming paradox of both genetic and environmental determination of weight (Carnell & Wardle, 2008a; Carnell et al., 2008).

In support of such a theory, it is known that genes influence a number of different behavioural and psychological traits as powerfully as physiological processes, including personality and IQ (Plomin et al., 2008). The idea that genes influence body weight through behavioural pathways such as appetite allows for those susceptible to the present obesogenic environment to gain the most weight, and recent studies have shown that the largest increases in BMI have occurred at the upper end of the continuum, consistent with this model (Wardle & Boniface, 2008). The diagram in Figure 2.2 illustrates, in a very simplistic schematisation, the dynamic interplay of genes and environment on eating behaviours in the development of weight. Importantly, there is a growing research literature focussing on the genetic influences on appetite and eating behaviour. This is described below.

Figure 2.1. Schematisation of a behavioural susceptibility model of weight



2.4. The heritability of appetitive traits

A number of studies have started to quantify the relative influence of genes and environment on appetitive traits using family and twin designs. The majority of them have focused on adults, so studies using either adults or children are therefore included in the summary of evidence below. Because family studies tend only to give an indication of 'familiality' which can include both genetic effects and those of the shared family environment, these are presented first followed by twin studies that are able to partition the effects more precisely into genetic, shared environmental, and unique environmental influences. A handful of studies have also attempted to establish if a number of different appetitive traits reflect the same underlying genetic pathway or are genetically distinct, by exploring the extent to which common genetic factors underlie multiple traits. These studies will be reviewed as well. Lastly, a couple of researchers have made efforts to establish genetic overlap between appetite or eating behaviours and measures of adiposity. These studies provide direct support for a behavioural susceptibility model of weight; the results will be discussed in relation to this hypothesis. (The findings from the studies in this review have been summarised in tables that may be viewed in Appendix 2).

A significant literature also exists surrounding actual food intake, taste perception (e.g. sweet sensitivity) or aversion to particular substances (e.g. phenylthiocarbamide and 6-n-propylthiouracil); these studies are not included here, rather the review includes studies related as closely as possible to the appetitive characteristics discussed in Chapter 1. Studies have also started to try and identify the specific genetic variants that may be involved but this literature is not included because the purpose of this review is to quantify the extent to which genetic influences on the whole may be involved in appetite regulation.

2.4.1. Family studies[10]

2.4.1.1. Responsiveness to food cues, sensitivity to internal satiety and emotional eating

Family studies have undoubtedly shown that relatives do tend to resemble one another in their eating behaviours, and this appears to be the case with children as well as adults. The familial basis of 'externality' in adults has been explored primarily using the TFEQ which has been administered to very large epidemiological family cohorts as part of ongoing studies, and attempts have been made to quantify the proportion of variance in the measures explainable by familial influences. In a French-Canadian sample of 202 families (*n*=684) from the Quebec Family Study, 18% of the variance in 'disinhibition' and 28% of the variance in 'hunger' was accounted for by 'familiality', while a much smaller proportion of the variance in the more cognitive 'restraint' scale came from influences shared by families (6%) (Provencher et al., 2005). Another large (*n*=624) and homogenous sample of 28 Old Order Amish families in the US (from the Amish Family Diabetes Study) reported a somewhat higher estimate for 'disinhibition' (40%), about the same for 'hunger' (23%) and a much higher proportion of variance explained by families for 'restraint' (28%) (Steinle et al., 2002). These studies suggest that 'hunger' and 'disinhibition' almost certainly have a familial basis which may be genetic, but the picture is unclear with regard to 'restraint'.

[10] See Appendix 2.1 for a summary table of the literature.

A few studies on a much smaller scale have explored how children's observed eating behaviour in the laboratory is associated with indicators of the same traits in their parents. Using this approach, significant associations have been reported for parent and child eating speed (bites/minute), meal duration and total caloric intake during a meal in 29 families with 18 month old infants (Agras et al., 1988). In addition, parental measures of external eating indexed using the TFEQ or DEBQ have been related to 'eating in the absence of hunger' in 3-6 year old children (*n*=75) (Cutting et al., 1999), lower compensation ability following a preload in 3-5 year olds (*n*=77) (Johnson & Birch, 1994) and child 'external eating' measured using the DEBQ-C in preschool children (*n*=142) (Jahnke & Warschburger, 2008). 'Emotional eating' in mothers also appears to be transmitted to preschool children, although only the association between mothers and sons was significant in a sample of 3-6 year olds as that between mothers and daughters was much smaller (0.29 and 0.14 respectively) (Jahnke & Warschburger, 2008).

Studies have also used sibling resemblance to identify 'familiality' of eating behaviour in children, and evidence for familial aggregation for energy intake and for 'eating in the absence of hunger' have been reported. Faith and colleagues (2004) used an energy compensation design with 32 sibling pairs aged 3-7 years to assess familial aggregation of total energy intake following preloads of differing energy content, and of the COMPX scores (see section 1.3.2.1. in Chapter 1 for an explanation of COMPX). They found significant sibling correlations for total energy intake (0.39), for percentages of fat, carbohydrate and protein intakes (0.66, 0.67, 0.61, respectively), but no significant familial aggregation for COMPX scores. Using a laboratory-based version of the 'eating in the absence of hunger' paradigm Fisher et al (2007) assessed sibling aggregation of both ad libitum dinner intake and intake in the absence of hunger in a much larger sample (*n*=801) of Hispanic children aged 4-19 years from 300 families from the Viva la Familia Study. About half of the variance in both 'eating in the absence of hunger' (51%) and 'meal energy intake' (52%) was explained by 'familiality' (Fisher et al., 2007).

2.4.1.2. Food preferences and neophobia

Food preferences and neophobia (similar to the concept of fussiness or pickiness but relates in particular to an individual's willingness to try unfamiliar foods) have also been explored. In keeping with the other eating behaviour traits, significant associations have also been observed between parents and children ranging from 2-17 years for questionnaire measures of 'neophobia' (Koivisto & Sjoden, 1996; Pliner & Loewen, 1997; Falciglia et al., 2004; Koivisto & Sjoden, 1997), and for questionnaire-based measures of food preferences between parents and children and among siblings (Logue et al., 1988; Pliner & Pelchat, 1986; Rozin, 1991).

2.4.1.3. Summary

It is clear that there is a familial aggregation of most of the eating behaviours that have been explored. However, correlations between parents and children (or between siblings) may suggest shared genes or shared environments because the two are confounded. Correlations between parents would provide evidence of a shared environment effect because parents are not biologically related (assuming that 'assortative mating'[11] is not in operation), and such associations have sometimes been observed for a few of these traits (Agras et al., 1988; Rozin, 1991; Koivisto & Sjoden, 1997; Logue et al., 1988). In addition, the associations between siblings are often higher than those between parents and children (Pliner & Pelchat, 1986), perhaps because siblings share more environmental factors in common that influence these traits. To complicate the matter more, correlations have sometimes been observed between parents and children but not between siblings, which could indicate a rearing influence or different uterine experiences (Pliner & Loewen, 1997).

As mentioned in relation to weight, another drawback with comparing parents and children is that behavioural phenotypes can change considerably over the lifespan (e.g.

[11] 'Assortative mating' refers to individuals choosing mates that are similar to themselves on genetically-determined traits, raising the possibility that unrelated spouses may have some genes in common. This concept is discussed in detail in Chapter 4.

Beauchamp & Cowart, 1987), as can the relative influences of genes and the environment (Bergen et al., 2007). Developmentally dependent expression of these traits can 'muddy' the picture of influences when individuals who are at different stages of development are compared, even if the sample are siblings reasonably close in age. These factors cannot be adequately accounted for in family studies, but twins offer a unique opportunity to explore genetic influences on these eating behaviours after taking account of the shared environmental effects, such that the magnitude of both influences may be quantified at the same time, whilst also controlling for the age of the children. Twin studies that have looked at these eating behaviours are discussed below.

2.4.2. Twin studies[12]

2.4.2.1.Responsiveness to food cues, sensitivity to internal satiety and emotional eating

Twin studies of the genetic and environmental influences on eating behaviour traits have produced mixed results with varying estimates of the genetic effect size from study to study. The heritability of the TFEQ has been explored in a few samples of adult twins. De Castro & Lilenfeld (2005) using 149 MZ & DZ pairs who had all lived separately for at least 1 year, found significant heritability for cognitive restraint (44%) and hunger (24%), but not for disinhibition (0%) which was accounted for by shared (40%) and unique (60%) environmental influences, while influences of the shared environment played no role for the other two traits (0% in each case). In contrast, another twin study using a cohort of 210 female adult pairs from the Virginia Twin Registry, and a modified 36-item version of the TFEQ, found heritability estimates of 45% for 'disinhibition' (with no shared environment effect, 0%) and 8% for 'hunger' (with a modest shared environment effect, 16%), but no significant genetic influence on 'restraint' (0%) which was accounted for entirely by shared environmental influences (31%) and those of the unique environment (69%) (Neale et al., 2003a). Tholin et al (2005) using a large cohort of 782 young male adult pairs from the Swedish Young Male Twins Register, and a modified 21-item version of the TFEQ (including three scales: 'cognitive restraint', 'emotional eating' and 'uncontrolled eating'), found comparatively high heritability estimates for 'cognitive

[12] See Appendix 2.2 for a summary table of the literature.

restraint' (59%), 'emotional eating' (60%) and 'uncontrolled eating' (45%) and no evidence of shared environmental influences on any of the traits. A more recent study that included (predominantly) female and male British and Finnish twins found similarly high heritability estimates for the traits, although estimates varied with sex and population: 'disinhibition' had the highest genetic influence for all sub-samples (45%-69%), 'restraint' ranged from 26%-63% (and estimates were higher in women than in men), and 'emotional eating' varied the most from less than 10% to about 45% (and was much lower in men than women); likewise the shared environment appeared not to play a role for any trait in any subsample (Keskitalo et al., 2008).

The heritability of the characteristics captured in the DEBQ have also shown modest genetic influence in a large study of 583 families which included twins and other family members; a quarter to a third of the variance in 'restraint' (31%), 'emotional eating' (25%) and 'external eating' (25%) was explained by genetic factors with no evidence of shared environmental influences (Sung et al., 2010). Furthermore, restrained eating measured using the 'restraint scale' has also been found to be importantly influenced by genes (43%) with no shared environmental effects in another large US-based twin study of males and females (n=1440) (Schur et al., 2008).

It is not clear why findings have been so varied with regard to psychometric measures of these eating traits in the adult twin literature. Sample size may be a factor as larger sample sizes are needed to give robust estimates, and it was only in the smallest sample that heritability for 'disinhibition' was estimated as 0% (de Castro & Lilenfeld, 2005), in all of the other studies genetic influence on this trait was modest to high. Sex differences are another possibility, and these have been reported previously in TFEQ scores (Neumark-Sztainer et al., 1999; Provencher et al., 2004). It is likely that the force of the environment on eating behaviour is different for females and males because of the pressure for women to stay slim – women may therefore battle against the tendency to overeat to a greater extent than men, in the attempt to conform to societal expectations (Neumark-Sztainer et al., 1999). Another observation is heterogeneity of samples which have included twins from the US, the UK, Finland, Sweden and Korea. It is likely that the cultural norms that govern eating will differ across these samples, affecting the dynamic interplay between genes and environment with regard to the expression of these behavioural traits. Of

consideration also is the heterogeneity of measures – no two studies of the TFEQ have used the same version.

Using adult studies to assess the heritability of eating behaviours also presents other problems. Firstly, the scales that have been used to ascertain genetic influences contain attitudinal (e.g. 'restraint') as well as behavioural components which may make it more difficult to pick up on simple genetic effects on the eating behaviours in question. This problem may be compounded in samples of adults who may have modified their eating behaviour or reports according to socially-prescribed attitudes. Secondly, studies with adult twins and families who have been living apart for a number of years may not detect shared environmental effects present in childhood which could have diminished by adulthood when children are living away from their siblings and parents (Koeppen-Schomerus et al., 2001). Lastly, some behavioural traits have shown profound change in the relative influence of genes and environment over time (Bergen et al., 2007).This makes it important to carry out paediatric studies as well.

As far as I am aware, only two twin studies have looked at comparable appetitive traits in children, one using a psychometric measure and the other using behavioural observation. The CEBQ was used to assess the heritability of 'satiety responsiveness'/'slowness in eating' (as a combined scale) and 'enjoyment of food' in 5435 twin pairs aged 8-11yrs from the Twins Early Development Study (TEDS) (Carnell et al., 2008). For 'satiety responsiveness'/'slowness in eating' model-fitting indicated heritability to be high at 63%, shared environment effects to be 21%, and non-shared individual environment effects to be 16%. For 'enjoyment of food', heritability was estimated to be even higher at 75%, shared environment to be slightly lower at 10% and non-shared environment to be 15%. When stratified by sex, heritability of 'enjoyment of food' was slightly higher for males than females (78% versus 70%).

The heritability of eating rate was explored fairly recently in the TEDS subsample (*n*=242 twin children) when the children were aged 8-11 years old (Llewellyn et al., 2008). Bites per minute consumed during a standard lunch of sandwiches and fruit at home were assessed, as described in the previous chapter. Heritability of eating rate was high at 62%, while non-shared environmental effects explained the remaining variance (38%) with no evidence of shared environmental influence. These heritability estimates observed in

children are high in comparison to many of those reported for similar characteristics in adults, indicating that eating behaviour in children is highly influenced by genetic factors, and raising the possibility that genes play a more important role in childhood than in adulthood for food responsiveness (the heritability of satiety sensitivity has not been assessed in adults).

2.4.2.2.Food preferences and neophobia

In contrast to studies of food responsiveness the picture with regard to food neophobia is much clearer – genetic influence on this trait is strong, and in contrast to the other characteristics, this appears to be the case for children as well as adults, although there may be sex differences. The full TEDS sample was also used to assess the heritability of neophobia when the twins were aged 8-11 years, using a parent-report questionnaire (*n*=5390); it was found to be a highly heritable trait (78%) with the remaining variance being accounted for by nonshared environmental effects (22%), and no evidence for any shared environment effects (Cooke et al., 2007). This finding has been replicated in two other large samples of Finnish and British adults for whom estimates of heritability ranged between 61% and 69% and shared environmental effects were 0% in each case (Knaapila et al., 2007; Knaapila et al., 2010). One important observation is that there were stark sex differences in one of the adult studies – individual differences in genetics explained the majority of the variation in neophobia for females but played no role for males (Knaapila et al., 2010), highlighting again that heritability estimates can vary greatly from one population to another. It is not inconceivable that the expression of this trait in adulthood is moderated to some extent by social norms – it may be less acceptable for adult males to be 'fussy' and cautious eaters, while women are permitted socially to be 'picky' with their food.

Twin designs have also indicated heritability for certain food preferences among children, although estimates appear to depend upon the type of food examined and findings have varied. A sub-sample of 214 male and female twin pairs from TEDS (aged 4-5 years) were used to estimate the heritability of food preferences using parents' reports for their children's liking of 77 different foods grouped into four catgeories using a factor analysis (Breen et al., 2006). Heritability estimates differed with food group type, with estimates of

0.20 for desserts, 0.37 for vegetables, 0.51 for fruits, and 0.78 for protein foods. Shared environmental effects were higher than those found for measures of food responsiveness, satiety sensitivity and neophobia described in the aforementioned studies, with 0.64 for desserts, 0.34 for fruits, 0.51 for vegetables, and 0.12 for proteins, suggesting that shared influences such as parental feeding styles or exposure to certain types of foods may increase liking for those foods in young children. It is also worthy of comment that MZ correlations were significantly higher than DZ correlations for 72 of the individual foods, indicating that there was a heritable basis to most of the separate foods.

Falciglia and Norton (1994) also assessed the heritability of food preferences ratings, but for individual foods rather than food groups, following a taste test of 17 foods in a sample of 35 twin pairs aged 9-18 years. They found significant differences in the intrapair correlations for only 6 of the 17 foods, including orange juice, broccoli, cottage cheese, chicken, sweetened cereal and hamburger. This was in keeping with Krondl and colleagues (1983) who found that one third of 24 individual foods examined had genetic influence, which also included orange juice and broccoli, as well as grapefruit juice, apple juice, strawberries, green beans and bacon. For these two studies the environment played a more influential role over liking than genes as for the majority of foods examined no genetic influence was detected.

Studies of the genetic influence on food preferences in adults have also yielded inconclusive results. A very early study by Faust (1974) explored twin resemblance for 'fads' and 'fancies' for individual foods, in a British sample of adults (n=192); no difference was found between MZ and DZ twins for any foods except for spicy foods. This finding was pretty well replicated in a later study of 72 twin pairs for whom resemblance for 13 individual foods and food spiciness was assessed (Rozin & Millman, 1987). In keeping with Faust's (1974) finding, the majority of the food preferences were equally correlated among MZs and DZs suggesting that the primary influence on food preferences was the shared environment, except for spiciness for which the MZ correlation was significantly higher (Rozin & Millman, 1987). On the other hand, a very recent and large twin study looked instead at preferences for high fat foods collectively (rather than individual foods) and found significant heritability of about 45% for liking and frequency of consumption (Keskitalo et al., 2008), although some sex differences were observed.

2.4.2.3. Summary

Only a handful of twin studies have ever explored the relative influences of genes and the environment on external food responsiveness and internal satiety sensitivity. In adults, all of the studies have used psychometric measures, but none has used behavioural observations. In spite of this, findings have varied hugely perhaps for some of the possible reasons discussed (sample and measure heterogeneity, and varying sample sizes). Nevertheless, larger studies that are sufficiently powered to detect smaller estimates reliably have found a modest role of genes for indices of external eating with heritability estimates explaining one quarter to one half of the individual differences observed (Keskitalo et al., 2008; Sung et al., 2010; Tholin et al., 2005), suggesting that genes play a reasonably important role in shaping the expression of these traits across a variety of adult populations. Similar or slightly higher estimates have also been observed across the larger studies for the more cognitive trait of restrained eating (Keskitalo et al., 2008; Schur et al., 2009; Sung et al., 2010; Tholin et al., 2005), although emotional eating appears to be a less stable finding with very large ranges in the estimates (9% to 60%) (Keskitalo et al., 2008; Sung et al., 2010; Tholin et al., 2005).

Only one study of food responsiveness could be identified for children (Carnell et al., 2008), although eating speed (Llewellyn et al., 2008) and satiety sensitivity have also been measured (Carnell et al., 2008). For all of these traits the estimates observed earlier in life were markedly higher (62% to 75%), suggesting that in childhood genes are important. Genetic effects can be time-limited, so it is possible that effects are stronger in childhood, and environmental pressures may be less (e.g. the pressure to be slim as an adult woman). Of course, none of the estimates can be directly compared to those of the TFEQ or DEBQ because of the different behaviours and measures used but it suffices to say that for children individual differences in genes explain a large proportion of the variance in the appetitive traits that have been measured. And these findings highlight the importance of looking at appetitive traits at all points during the lifespan in order to understand how genes and environment may be conspiring in the development of appetite.

For food neophobia, genes appear to be playing an important role throughout the lifespan, although potentially more so for females than for males in adulthood, suggesting that

genes can interact with sex in moulding the expression of eating behaviour and it is important to take this into account when exploring influences within any given sample. 'Food neophobia' is considered a stable trait (Pliner & Hobden, 1992) and has also been likened to certain dispositional personality characteristics such as 'emotionality' (Pliner & Loewen, 1997) and 'anxiety' (Pliner & Hobden, 1992), which are themselves moderately influenced by genes (Eid et al., 2003; Legrand et al., 1999). This trait is therefore likely to tap non-appetitive domains as well, and may reflect genes that do not solely discriminate one's approach to new foods but may influence vigilance more generally, and this trait has been shown to covary negatively with 'openness', novelty-seeking and excitement-related dimensions (Knaapila et al., 2010; Pliner & Hobden, 1992).

Food preferences appear to represent a slightly different case to the aforementioned eating behaviour characteristics. There is some evidence of genetic influence on liking or preference for certain types of food, but the size of the effect depends on the kind of food examined (Breen et al., 2006), and liking of groups of food (e.g. fatty foods) seems slightly more heritable than liking of individual foods (e.g. Breen et al., 2006; Keskitalo et al., 2008) (versusFalciglia & Norton, 1994; Krondl et al., 1983; Faust, 1974; Rozin & Millman, 1987). This is not surprising given that aggregation of individual foods into groups accounts much better for individual deviations from the 'general rule', in the same way that psychometric measures of traits such as satiety sensitivity get around the issue of temporary extraneous factors clouding measurement of the broader underlying trait. Even so, heritability is not strikingly high for preferences for food groups across the board for children, and this contrasts with the other appetitive traits that have been measured. Early reviews on food preferences concluded that genes play a relatively unimportant role in shaping these characteristics in comparison to environmental factors, but that they are nevertheless involved to some level (Cavali-Sforza, 1990; Perusse & Bouchard, 1994; Reed et al., 1997). It makes sense for there to be considerable room for environmental influence on food preferences given the need for human beings as omnivores to be able to eat a range of foods, and adapt their tastes to match availability; in support of this, Rozin (1976) pointed out that it is a characteristic feature of omnivores to have few biological predispositions that govern food choice, and those that are present tend to influence choices across the board, such as the universal liking for sweet tastes.

2.4.3. Genetic overlap[13]

2.4.3.1. Shared genetic pathways underlying appetitive traits

A few studies have provided some evidence that the same genetic influences that underlie one appetitive trait also influence another. Such common genetic influence, termed 'pleiotropy' (one gene influencing more than one phenotype), suggests that the characteristics measured represent different manifestations of the same underlying genetic pathway. Studies can look at this in two ways: firstly, genetic or environmental correlations can be calculated between traits which indicate the extent to which common genetic effects are influencing both traits – a correlation of 0 indicates that none of the genes are the same, while a correlation of 1.0 would suggest that all of the genes underlying both are the same; secondly, the observed correlation between the traits (the 'phenotypic' correlation) can be partitioned into the proportion explained by common genes underlying the two characteristics, and the proportion accounted for by common environmental influences. These methods are described in detail in Chapter 4.

Fisher and colleagues (2007) who explored the familiality of energy intake and 'eating in the absence of hunger' among hispanic children, also considered if common familial influences underlie these two characteristics. The familial correlation (including common genetic and shared environmental influences) observed between these two behaviours was not significant, with or without adjustment for BMI, suggesting that the phenotypic correlation observed between the behaviours was not being driven by genes common to both (or shared environmental influences that give rise to both). However, extremely large sample sizes are needed to detect these correlations reliably and the sample may not have been sufficient to enable this (n=801); developmental differences between the siblings may also have created 'noise' making it difficult to detect genetic associations.

In contrast, two twin studies in adults have reported significant genetic correlations between some of the traits of the TFEQ. Neale and colleagues (2003a) found that 'disinhibition' and 'hunger' were significantly correlated (0.79), and that just over one third

[13] See Appendix 2.3 for a summary table of the literature.

of the genetic effects that influence the two traits are in common as suggested by a moderate genetic correlation (0.39). Common genetic effects explained about 40% of the covariance between these two traits. 'Restraint' was not correlated with the other characteristics in this particular study. Keskitalo et al (2008) also found significant genetic correlations between the traits of the TFEQ, but the traits measured were slightly different making it difficult to compare findings across the two studies. Nonetheless, 'emotional eating' was phenotypically correlated with both 'uncontrolled eating' (0.56) and 'cognitive restraint' (0.24) and the corresponding genetic correlations were of a modest to large magnitude (0.75 and 0.42) suggesting that the majority of the genes that relate to 'emotional eating' also influence 'uncontrolled eating', and almost half of the genetic influences that influence 'emotional eating' and 'cognitive restraint' could be the same. Moreover, the observed association between 'emotional eating' and 'cognitive restraint' was entirely explained by common genetic influences, while the common genes accounted for just over half of the phenotypic association between 'emotional eating' and 'uncontrolled eating'.

Together the findings from these twin studies provide compelling evidence that a number of different appetitive characteristics arise from a common genetic pathway, raising the possibility that there are a set of genes that are important in modulating the expression of appetitive traits up or down, on the whole. This makes it seem likely that appetitive traits to some extent are different manifestations of the same underlying pathway rather than physiologically distinct phenomena. No studies have ever explored directly whether measures of satiety sensitivity (reflecting homeostatic appetitive control) and food responsiveness (more indicative of hedonic processes) share a common genetic pathway, and no study has ever examined shared pathways between eating behaviours during early life when considerable neurological plasticity is present.

2.4.3.2. Shared genes underlying appetitive traits and adiposity

Only two studies (a family study and a twin study) could be identified that explored commonality in the genes underlying any of these appetitive traits and adiposity. Fisher and colleagues (2007) reported significant 'familial' (genetic and shared environmental)

correlations between three measures of adiposity (BMI, fat mass and fat free mass) and dinner intake, although none of the familial correlations between indices of adiposity and 'eating in the absence of hunger' were significant. This could be due to the limitations of family studies, discussed earlier.

Keskitalo et al (2008) reported significant genetic correlations between factors of the TFEQ and BMI in adult twins. 'Cognitive restraint' and BMI had a small genetic correlation (0.16), but the phenotypic association (0.13) was entirely explained by these common genes; 'uncontrolled eating' and BMI had a modest genetic correlation (0.29) and shared genes explained the majority of the phenotypic association between the traits (81%); 'emotional eating' and BMI had a modest to high genetic correlation (0.51) suggesting that about half of the genetic effects underlying the two traits were the same, and again the majority of the phenotypic association between the two traits was explained by common genetic influences (81%). This study suggests that there is a common pathway underlying these appetitive traits and weight. Of course, it could be the case that the genes that influence weight ultimately influence appetite because weight influences appetite, but the other reverse scenario is just as likely. Nevertheless, finding common genes underlying appetite and weight provides positive evidence in support of a behavioural susceptibility model of weight. However, this area of research is largely unexplored.

2.5 Conclusions and future research

There is a great deal of interest in understanding the genetic architecture underlying weight, and the processes behind rapid weight gain during very early life. Chapter 1 provided evidence for the causal role that appetite may play for the development of adiposity in children and infants, such that those with more voracious appetites are at greater risk for excessive weight gain. This chapter briefly described the neurological and physiological mechanisms that govern appetite through homeostatic and hedonic processes, making it possible that genetic variants related to the biology of appetite might cause individual differences in appetite avidity, and ultimately cause variation in adiposity reflecting these traits. A behavioural susceptibility model of weight whereby inherited individual differences in genes influencing appetitive processes make some individuals more susceptible than others to the temptations of the current 'obesogenic' environment

allows for both genetic and environmental determination of weight, and places appetite on the causal pathway.

A fairly substantial evidence base exists to support the idea that individual differences in a number of eating behaviours may be partly explained by genetic differences, although far less work has been carried out in children and no-one has explored genetic influences on appetite during infancy. Very little work has been done to explore shared pathways underlying appetitive characteristics and weight, although evidence of a shared causal pathway would go a long way towards making the case for the behavioural susceptibility model of weight. Another question of interest, with little existing literature, is the extent to which different eating behaviours represent the same underlying genetic causes, or whether they are independent phenomena. The need to understand how genes influence appetite from early in the life cycle, and for more complex studies of shared pathways, are discussed in more detail below.

2.5.1. A need for early life studies of genetic influence on eating behaviours

There is reasonable evidence for genetic influence on several eating behaviours in adults including those indicative of food responsiveness such as ‘disinhibition’ and ‘hunger’ as measured by the TFEQ. However, findings have varied hugely in adult populations not only as a result of the different types of behaviours measured, heterogeneity of measurement tools, and diverse sample characteristics, but probably also due to the numerous other social and psychological processes known to influence eating behaviour in adults. Another observation is that estimates often differ for males and females, probably as a result of different environmental pressures.

Studies of food responsiveness, satiety sensitivity and eating speed have shown high genetic influence in children (62%-75%), although the heritability of food preferences is probably lower and estimates vary with food type. However, on the whole very few studies have been conducted during childhood and none has explored the influence of genes on appetitive traits during infancy. There are important reasons for measuring the genetic influence on appetite avidity in very early life. Firstly, heritability estimates can change over the life span making it important to measure traits at key stages during development.

Secondly, Chapter 1 highlighted the importance of infancy as a critical period for 'programming' later obesity risk. If appetitive characteristics play a role in facilitating rapid weight gain their aetiology must be properly understood so that interventions can be designed to attenuate their expression, in-line with sound theoretical knowledge to increase the likelihood of their success.

There are other reasons why studies during infancy are required. The influence of the shared family environment is arguably much more important during early life than it is in adulthood as parents play a central role in permitting the expression of these traits in their infants and children; on the other hand, adults are able to make independent decisions about their eating behaviour in-line with their own genetic proclivities without so many constraints. Using adult twins or family members who have been living apart for a long period of time may not detect these important shared effects that are stronger during the early life period. An exploration of the relative influences on eating behaviours during the formative years may therefore provide more insights into this aspect of the environment.

A final consideration is that very 'clean' measurement of genetic effects on eating (or feeding) behaviour may be possible during very early infancy. During this period of life the environmental influences are limited to immediate physiological processes such as current hunger state (i.e. time since last feed), ambient temperature, or noise, while social and psychological influences would not yet play a role. Use of a psychometric measure of appetitive characteristics for infants would minimise the influence of these even further as parents would be able to average their baby's feeding behaviour over many different occasions. As mentioned in Chapter 1, no such measure for infancy currently exists.

Lastly, and in relation to the point above, very large samples are needed to provide robust and reliable estimates of heritability. Inadequate sample sizes may go part way in explaining the variability of estimates seen in the adult literature. Assessing heritability through behavioural measures limits power to detect effects as these studies are necessarily smaller, whereas psychometric measurement allows for large-scale quantitative genetic analysis to be carried out with relative ease. The CEBQ has made it possible to establish with some reliability the heritability of key appetitive traits in a very large sample of children. Assessment of these traits using a similar measure in a very large sample of infants would contribute to the existing knowledge-base.

2.5.2. A need to understand the shared genetic pathways underlying eating behaviours in early life

A question that has not been addressed adequately in the literature thus far is whether these eating behaviours represent different aspects of the same underlying trait and originate from one genetic causal pathway, or whether they have different genetic aetiologies and are in essence independent entities. Only two twin studies could be identified that have explored genetic associations between appetitive traits and both provided some evidence that there are common genes involved. However, the studies were conducted in adults, and limited traits were investigated, including 'disinhibition' or 'uncontrolled eating' and the more psychological or cognitive traits of 'restraint' and 'emotional eating'. However, shared genetic pathways that underlie the more homeostatic trait of satiety sensitivity and the more hedonic traits such as food responsiveness or enjoyment of food have never been explored. This question is of interest given the growing research base that is showing considerable cross-talk between the homeostatic and hedonic systems that control appetite. Another question that remains to be answered is how these appetitive characteristics relate to one another during very early life, a period of development that is characterised by considerable neurological plasticity. It may be the case that the genetic architecture underlying appetite in very early life is different to that later on, not only because of early plasticity, but also because genetic effects can be time-limited. It would be useful to explore shared genetic pathways underlying appetite during infancy.

2.5.3. A need to understand shared genetic pathways between appetite and weight in early life

A number of these eating behaviours relate to weight in children, and some studies have suggested a causal role, as discussed in Chapter 1. In children, weight and eating behaviours both have a genetic basis, and it has been proposed that inherited individual differences in appetite avidity would help to explain the seeming paradox of both genetic and environmental determination of weight. If eating behaviours (at least partly) mediate genetic influences on weight, then eating behaviours and weight should show a shared genetic pathway. Only one adult twin study has ever looked at this issue, using a limited

number of appetitive characteristics. ‘Cognitive restraint’, ‘uncontrolled eating’ and ‘emotional eating’ measured using the TFEQ appeared to share some genes with BMI. It would be interesting to identify if the key appetitive trait of satiety sensitivity shares common genetic influences with weight. Moreover, because of the importance of rapid weight gain during infancy for later obesity risk, and the evidence that individual differences in appetitive traits are present from the beginning of life and linked to adiposity, it would be prudent to identify shared genetic and environmental pathways underlying appetite and weight as early on as possible to help shed light on the processes by which excessive weight is gained so that it might be prevented. Finding evidence of shared genes underlying appetite and adiposity would contribute meaningfully to the evidence base and would provide further support for a behavioural susceptibility model of weight. No study has yet explored if any appetitive traits share genes in common with weight early in life.

CHAPTER 3. RESEARCH AIMS OF THE CURRENT THESIS

3.1. Aims and outline of the research in this thesis

The first chapter of this thesis provided evidence for associations between appetitive traits and weight during infancy and childhood, and evaluated the likelihood that appetite plays a causal role in the development of adiposity. In Chapter 2, the case for a behavioural susceptibility model of weight was presented, along with the evidence base for genetic influence on appetitive traits in children and adults. In each review limitations of the existing literature were highlighted, which included little or no research in the infancy period for either research base. The overall aim of the current thesis is to test one of the assumptions of the behavioural susceptibility model of weight, namely that inherited differences in appetite are already present in infancy, and that shared genetic effects contribute to the associations between appetite and weight from early in life. In order to establish this, I undertake a number of studies to help to piece together the necessary evidence. A secondary question that is addressed is the extent to which the different appetitive traits share a common genetic pathway. The studies in this thesis are outlined below.

3.1.1. The development of a standardized psychometric measure of appetitive traits during infancy

Very large-scale studies are needed to detect small associations between appetitive traits and weight, or to establish heritability estimates reliably. Observational measures of appetite, however, can only be used in smaller samples and may fall victim to the extraneous factors at play at the time the behaviour is observed. The CEBQ provides a reliable and standardized psychometric method of measuring appetitive traits in children that has permitted large-scale research to be conducted to establish both relationships with weight and heritability. No such tool exists for the infancy period. What is more, very little work has been done to characterize the dimensions underlying infant appetite, to explore associations with weight during the first few months of life, or to uncover the genetic influences on appetite during this early period. A standardised psychometric

measure of appetite during infancy, comparable to the CEBQ, would allow large-scale research to be carried out to establish relationships with weight and heritability from the beginning of life. Study 1 (Chapter 6) of this thesis describes the development and factor structure of such an instrument for the very earliest period of life when infants are still exclusively fed milk – the Baby Eating Behaviour Questionnaire (BEBQ). This instrument enables me to demonstrate that there are individual differences in appetite that are present and measurable from early in life, and provides a suitable instrument for establishing heritability and associations with weight.

3.1.2. Establishing relationships between appetitive traits and weight in infancy

There has been a lot of recent interest in understanding the processes that drive rapid growth in infancy, and the spotlight has turned on appetite as a potential driver of weight gain. Few studies have been carried out to explore how appetite avidity relates to weight during infancy. However, there is reason to believe that large individual differences in appetitive traits are present from the first few days of life, and these may play a role in mediating variation in early growth rates. Study 2 (Chapter 7) uses the BEBQ to explore how appetitive traits assessed during the first few months of life relate to weight.

3.1.3. Establishing the heritability of appetitive traits in infancy

Studies have shown that genetic differences help to explain individual differences in appetite potency in adults and children, but the relative influences of genes and the environment on appetitive traits have never been explored in infancy. Given that genetic expression is often age-dependent, heritability during early life cannot be assumed. If inherited individual differences in appetite avidity that are present from the beginning of life are driving the associations with weight and contributing to the heritability of weight, they themselves should show a reasonable level of genetic influence during this early period. Study 3 (Chapter 8) establishes the relative influences of genes and the environment for the appetitive traits captured in the BEBQ.

3.1.4. Identifying shared genetic pathways underlying appetitive traits in infancy

A question that has been of some interest in the literature is the extent to which different appetitive traits such as satiety sensitivity and food responsiveness arise from the same underlying genetic pathway, or are genetically independent. Neurological research has made some headway in showing interactions between homeostatic and hedonic systems governing food intake, but a very limited amount of research has been conducted into the shared genetic pathways underlying appetitive traits, with few traits investigated and only in adults. Study 4 (Chapter 9) explores the extent to which the traits captured in the BEBQ share a common genetic pathway.

3.1.5. Identifying shared genetic pathways underlying appetite and weight during infancy

If inherited individual differences in appetite from the beginning of life are driving the association with weight and contributing to weight heritability, appetite and weight should show shared genetic pathways, and common genetic influences should contribute importantly to the phenotypic association between the two. Study 5 (Chapter 10) provides the final piece in the puzzle by exploring shared genetic and environmental pathways underlying appetite and weight in early life.

3.1.6. An in-depth exploration of an infant with a highly avid appetite

Studies 1 to 5 use quantitative methods to test if the assumptions of a behavioural susceptibility model are consistent with the evidence in the early life period. While quantitative methods are necessary for hypothesis-testing, the participant numbers needed for sound statistical testing limit the amount of information and context that can be deemed for each individual included. In-depth study of extreme cases can enrich quantitative findings by providing a detailed analysis of the characteristics of interest to increase understanding and knowledge of those traits. In relation to appetite, it enables me to study and describe the eating behaviours that characterise a highly upregulated appetite, and to engage in a full exploration of their likely origin as well as the ‘real life’

consequences for the infant and the family in terms of weight gain and parental management. Study 6 (Chapter 11) provides an in-depth exploration of a single case of an infant with extreme appetitive avidity whose parents were forced to exert drastic control measures to avoid severe overeating.

3.2. Samples

In order to achieve these aims I use data from a large population-based birth cohort of infant twins, Gemini – Health and Development in Twins. The size of the sample enables me to establish reliable associations between appetite and weight, the twin design allows me to explore genetic and environmental influences on appetite and weight, and the young age of the sample permits me to test some of the assumptions of the behavioural susceptibility model during the early period of life. Details about the sampling methods and measures taken in Gemini are described in detail in Chapter 5. A single case of an infant with extreme appetitive avidity allows me to explore how a highly upregulated appetite manifests itself during early life.

3.3. Quantitative genetic analysis

The quantitative genetic methods used to establish the heritability of traits, as well as shared genetic pathways underlying multiple traits, are complex and have many assumptions. They are described in detail in Chapter 4.

3.4. My contributions to the research included in this thesis

I have been involved in the Gemini study since it was first set up by Professor Jane Wardle in 2007. I attended the early meetings with the Office for National Statistics to assist with recruitment procedures, was involved in putting together the participant information leaflets, invitation letters and consent forms, and played an important part in the choice or design of all the questionnaire measures, and pilot work for the study. I took personal

responsibility for the collection and management of DNA for the zygosity testing (described in Chapter 5). Together with the other team members, I sent out postal questionnaires and entered some of the data. Dr Ellen van Jaarsveld, the study co-ordinator (and my second supervisor) cleaned the data. All the analyses in this thesis were performed by me unless indicated by footnotes, and I came up with the overall thesis aim and designed all the analyses that allow me to answer my questions.

The most significant contribution I have made is to learn how to do quantitative genetic modeling, by attending a 1-week course at the MRC Social Genetic and Developmental Psychiatry Centre at the Institute of Psychiatry, Kings College London, in July 2009. I found this process extremely challenging. The methods used are very complex, and few researchers in this country use these modeling techniques. The course taught me the basic principles and introduced me to the software used; immediately following the course I was only able to run simple univariate models. I taught myself the methods for running the more complex models by studying the course booklet, reading papers that had used complex models, and joining and attending genetics journal clubs at the Institute of Psychiatry and Birkbeck in order to forge relationships with other researchers working in this field. I am now able to write my own scripts for most of the complex models (a requirement), and can interpret the output with ease. This skill enabled me to identify new methods for answering novel questions relating to the behavioural susceptibility model of weight, such as whether appetite and weight share common genetic influences.

CHAPTER 4. QUANTITATIVE METHODS FOR STUDYING THE GENETIC AND ENVIRONMENTAL INFLUENCES ON BEHAVIOURAL TRAITS

This chapter outlines the methods used in quantitative genetic model-fitting to estimate the relative importance of genes and the environment on given traits using twins. Basic laws of heritability are briefly discussed first, in order to provide the background theory upon which the statistical methods are based. The chapter then provides detailed information about the statistical methods used to model twin data. Lastly, other complexities that may be incorporated into model-fitting are discussed. Much of the information provided in this chapter has been taken from a book on quantitative genetics by Robert Plomin and colleagues (Plomin et al., 2008), and the lectures and course book from the 10th MRC SGDP Centre Twin-Modelling Summer School held at the Institute of Psychiatry, Kings College London, which I attended from July 13th to July 17th, 2009 (Institute of Psychiatry, 2009).

4.1. Study designs – the usefulness of twins

At the heart of Quantitative Genetics Theory (QGT) is the assumption that the observed variance of any given trait reflects unobserved latent factors which include genetic effects and environmental effects (the latter include any effects not directly caused by the functional effects of genes). The environmental effects may be further broken down: (1) the shared environment effects which include all aspects of the environment that are both shared by relatives, and that make relatives more similar on a given trait (these could include going to the same school, being the same age, or two siblings being treated the same by their parents); (2) the non-shared or 'unique' environment which includes all effects of the environment that make relatives different from one another (such unique experiences could include illness, unique friendships, or being treated differently by parents). QGT uses samples of individuals who differ in their genetic relatedness to make predictions about the relative effects of the underlying genetic and environmental factors on a given measurable characteristic.

A number of different designs can be used to disentangle the effects of genetics and the environment, but some offer more insight than others. Families (e.g. parents and offspring, full siblings, half siblings, cousins, etc) are often the starting point for inferring genetic influence on a trait or disease. If a relative of a person with a particular disease is more likely to develop that disease than a relative of a person without that disease, then it is possible that it has a heritable basis (Plomin et al., 2008). Nonetheless, family disposition is not sufficient to assume genetic aetiology because families may share predisposing environments as well as genes, so correlations among family members simply give an indication of 'familiality' which needs to be broken down into shared environmental risk factors and genetic risk factors (Martin et al., 1997).

Adoption studies offer a solution to this problem – adopted children and their adoptive parents, or two adopted and biologically unrelated siblings, only have their shared environment in common (because they are not genetically related) so any increased familial risk for a disease must be bestowed through aspects of their environment that are shared (Plomin et al., 2008). The reverse adoption situation also offers insight into genetic influences – if a child who is adopted away from their biological parent (or adopted away full biological siblings) has as high a risk of developing a disease afflicting their biological parent as a child who is reared by their biological parent (or two full siblings reared together), then it is likely that inherited genes are causing the association, as the parent and child (or adopted away siblings) are assumed to have no environmental factors in common (Plomin et al., 2008). One potential limitation of this design is that adoption agencies generally attempt to place children with families that are matched to the physical, behavioural and cultural attributes of the biological parents, which potentially introduces the possibility that there are some shared environmental factors across the two households (Hardy-Brown et al., 1980). Another drawback of this design is that adoptions are becoming increasingly rare and atypical as a result of modern contraceptive options (Martin et al., 1997); aside from the difficulties of sufficient data collection, biological and adoptive parents and adopted children are not representative of the population at large compromising the generalisability of the results. In addition, data on birth parents are rare (Plomin et al., 2008).

Monozygotic twins (MZs) or 'identical' twins are so-called because they develop from one zygote (fertilised egg); they are therefore genetically identical and so share 100% of their

DNA. This being so, MZ twins reared apart also offer the opportunity to evaluate the influence of genes alone on a trait – any correlation between MZs reared apart is assumed to be due entirely to genetic influences as no shared environments are contributing (Plomin et al., 2008). However, this design is plagued by the same drawbacks as adoption studies – cases of these are extremely rare and the children are often placed with similar families opening up the possibility of some shared environmental influences contributing to similarity (Jospeh, 2004).

The combination of MZ twins and dizygotic (DZ) twins offers a powerful and convenient design for untangling the genetic from the environmental influences on characteristics of interest; moreover, this design has benefits over adoption studies and MZ twins reared apart. While MZ twins come from one zygote and share 100% of their DNA, DZ twins (or 'non-identical' or 'fraternal' twins) develop from two zygotes and only share on average 50% of their genetic information, like full siblings. At the same time, both MZs and DZs are assumed to have the same type of shared environmental experiences – e.g. they are both the same age, and are brought up within the same family, etc. So, any within-pair difference between MZ similarity and DZ similarity is assumed to be due solely to differences in the amount of shared genetic information – specifically, if a trait is genetically determined MZ pairs must be more similar than DZ pairs (Plomin et al., 2008). This makes it relatively easy to estimate the importance of genes and the environment for any trait of interest.

In addition, almost every characteristic studied varies with age, and genes can be time-limited in their expression; because twins are matched for age, familial and social influences to a far greater extent than are regular siblings or parents and off-spring, it is easier to interpret the genetic and shared environment effects from twin studies than those found in adoption studies (Martin et al., 1997). DZs are also much less likely to have different biological fathers than regular siblings (Martin et al., 1997). Of importance as well is the preponderance of twins. MZ and DZ twins crop up in regular proportions across many populations making them a fairly accessible sample for collecting large amounts of data. The statistical modelling methods for estimating the relative effects from genes and the environment will be explained in detail in section 4.5 below. Firstly, some basic principles of genetics upon which the models are based will be explained.

4.2 Alleles and genotypes

To illustrate how twins can be used to identify the relative influences of genes and the environment on measured characteristics or 'phenotypes', the example of a single gene will be used as this forms the basis of the methods for 'complex' traits (traits that arise from a combination of multiple genes). At any given locus an individual carries two alleles that constitute their genotype – one allele is inherited from their mother and one from their father. Assuming that only two forms of the gene exist in the population (A_1 and A_2) there are three possible genotypes: individuals who have two A_1 alleles (A_1A_1) or two A_2 alleles (A_2A_2) are termed 'homozygous' and individuals who have one A_1 allele and one A_2 allele (A_1A_2) are termed 'heterozygous'.

4.3. Genetic effects – additivity and dominance

The combined effects of the two alleles at any given locus on a trait may be described as 'additive' or 'dominant', depending on their expression in the heterozygous genotype (Plomin et al., 2008). If the combined effects of the two alleles are equal to the sum of their individual effects, the genotypic effect is said to be 'additive', and the heterozygote's genotype value will be intermediate to the two homozygous genotype values – e.g. if the value of A_1 is 0, and the value of A_2 is 1, the genotypic value for the two homozygotes will be 0 ($A_1 + A_1$) and 2 ($A_2 + A_2$), while the heterozygote will have an intermediate value of 1 ($A_1 + A_2$) (Plomin et al., 2008).

On the other hand, genetic dominance is present if one allele is expressed more fully than the other when two different alleles are present at the same locus, i.e. the expression of one allele depends on the other allele present at that locus (also referred to as a biallelic interaction). To illustrate this further using a simple example with complete dominance, if A_2 has a value of 1 but A_2 is never expressed in the presence of A_1, the heterozygote's genotypic expression will be 0 (as opposed to 1, in the additive case), and the A_1 allele is said to be 'dominant'. Because the genotypic expression gives rise to the phenotype (directly or indirectly), if dominance effects are present the phenotype of the heterozygote

will bear more resemblance to one homozygote than the other. An example is the genotype for a cleft chin – homozygotes for the cleft chin allele (CC) and heterozygotes with an allele for a cleft chin and an allele for no cleft (Cc) are phenotypically indistinguishable, while the allele for no cleft chin is only expressed in individuals homozygous for the no cleft chin allele (cc) (Lebow & Sawin, 1941). Dominance can be partial or complete, and the 'dominance deviation' is the gap between the observed genotypic values and the predicted values for an additive model (Plomin et al., 2008).

Additive and dominant genetic effects are transmitted differently from parents to offspring, and siblings are correlated differently for both as well. A key concept underlying additive and dominance inheritance is being 'identical-by-descent' (sharing exactly the same allele inherited from the same mother or the same father) rather than identical by phenotype. Children receive half of all of their alleles from their father and the other half from their mother, so they share 50% of their genetic information with each parent. All of the additive effects inherited from each parent will be expressed in the offspring (i.e. off-spring share 50% of each parent's additive genetic effects) because each additive allele will be expressed in the same way in the offspring as in the parent. In addition, because full siblings share the same two parents there is a 50% chance that two siblings will share one allele that is identical-by-descent (i.e. the same allele from the mother or father); a 25% chance that two siblings will be identical-by-descent on both alleles (i.e. that they will inherit the same allele from each parent); a 25% chance of sharing no alleles that are identical-by-descent (these probabilities are illustrated in Table 4.1). In this way, full siblings also share on average 50% of their additive genetic effects with one another, because there is a 50% chance that they will share 1 allele out of 2.

In the case of genetic dominance a genotypic value depends upon the exact combination of alleles at that locus. As children only inherit one allele from each parent (rather than the specific combination within each individual parent that gives rise to the mother's or father's expression), genetic dominance is not transmitted from parent to off-spring. On the other hand, there is a 25% chance that two siblings will be identical-by descent for two parental alleles, i.e. that they will inherit the same allele from their mother and their father, thereby having the same combination of alleles at that locus; so genetic dominance is correlated in full siblings by 0.25. The probabilities of two full siblings being identical-by-descent for 0, 1

or 2 alleles are presented in Table 4.1 for two (maternal and paternal) alleles (the table has been adapted from one presented in the Twin Model-Fitting Course Booklet for the 10th MRC SGDP Centre Summer School, (Institute of Psychiatry, 2009)).

Identical twins, being genetically identical, share 100% of both their additive and dominance genetic effects in comparison to full siblings sharing just 25% and parents-offspring sharing none. So the hallmark of genetic dominance is that first-degree relatives (parents-offspring or full siblings) are less than half as similar in comparison. Table 4.2 shows the amount of additive and dominance genetic effects shared by different relatives within a family.

Table 4.1. Four-by-four matrix showing all 16 possible combinations of two parental alleles (Mm and Pp) at a locus for two full siblings (table adapted from Institute of Psychiatry, 2009)

Sibling/sibling parental alleles (number of alleles that are identical-by-descent)				
	MP	**mP**	**Mp**	**Mp**
MP	**MP/MP (2)**	MP/mP (1)	MP/Mp(1)	MP/mp (0)
mP	mP/MP (1)	**mP/mP (2)**	mP/Mp (0)	mP/mp (1)
Mp	Mp/MP (1)	Mp/mP (0)	**Mp/Mp (2)**	Mp/mp (1)
mp	mp/MP (0)	mp/mP (1)	mp/Mp (1)	**mp/mp (2)**

At a biallelic locus there are four possible combinations of maternal (Mm) and paternal alleles (Pp) in the offspring: MP, mP, Mp, mp. Each of two children will have four possible combinations of inheritance, giving rise to 4*4 (16) possible sibling-sibling combinations for two full siblings. The number of alleles that are shared by each combination are shown in parentheses. ¼ of the sibling-sibling combinations will be identical-by descent for both alleles (two siblings inherit the same allele from each parent resulting in the same combination of alleles in the two siblings), shown above in bold – MP/MP, mP/mP, Mp/mP, mp/mp. ½ of the sibling-sibling combinations will be identical-by-descent for one parental allele (two siblings inherit the same allele from one parent, but have different alleles from the other parent) – e.g. MP/mP. ¼ of the sibling-sibling combinations share no parental alleles in common – e.g. MP/mp.

Table 4.2. Coefficients of genetic relatedness for different biological family members

Pair of biological relatives	Proportion of additive genetic variance shared	Proportion of dominance genetic variance shared
Adoptive parents and adopted children	**0%**	**0%**
First degree cousins	**12.5%**	**0%**
Great-grandparent and great-grandchild	**12.5%**	**0%**
Great-uncle/aunt and great-nephew/niece	**12.5%**	**0%**
Grandparent and grandchild	**25%**	**0%**
Half Siblings	**25%**	**0%**
Uncle/aunt and nephew/niece	**25%**	**0%**
Parent and offspring	**50%**	**0%**
Full Siblings	**50%**	**25%**
Fraternal twins	**50%**	**25%**
Identical twins	**100%**	**100%**

4.4. Polygenic traits

The large amount of variation observed for complex traits such as weight or appetite have led researchers to hypothesise that it is likely that multiple genes are influencing them, rather than just a single gene (Plomin et al., 2008). It has been proposed that the combined additive effects of many genes conspire to create a continuously distributed trait, with each allele contributing a small amount towards the phenotype. Environmental influences create even greater variation. So, multiple genes (and environmental effects) are probably combining to create normally distributed traits such as weight.

In terms of modeling genetic and environmental effects on polygenic traits, the same rules that apply to single-gene effects apply also to multiple gene effects. Additive genetic effects and dominance effects may be summed across all the loci influencing any given trait, and estimated using quantitative genetic model-fitting methods, detailed below.

4.5. Estimating genetic and environmental effects for a single phenotype using twins

'Heritability' (h^2) is the statistic that is used to describe the genetic effect size and it indicates the proportion of phenotypic variance that is explained by individual differences in genes rather than individual differences in environmental influences. The h^2 estimate ranges from 0-1 with a score of 1 indicating 100% heritability for a trait (and no influences of environmental factors), or a score of 0 suggesting that only environmental factors are at play. Importantly, h^2 indicates the contribution of different genotypes to individual differences, not to one person's characteristic or trait (Sesardic, 2005). There are two types of heritability: 'broad sense' h^2 includes all genetic effects (additive and dominance) thus indicating the extent to which genetic factors on the whole influence individual differences in a population; 'narrow sense' h^2 only includes variance accounted for by genotypic variation that is additive (Plomin et al., 2008).

In order to estimate heritability, quantitative genetics uses the variance components approach (Plomin et al., 2008). Central to the method is the assumption that the variance of a phenotype [P] comprises all genetic effects on the trait (additive and dominant) [G] and all environmental effects on the trait (shared and non-shared) [E]. The goal of quantitative genetics is to partition the variance of the phenotype of interest in to its constituent parts. The essence of the method is to contrast the observed phenotypic covariance among relatives, with what would be expected given their level of genetic relatedness. In order to do this, specific constraints are imposed on the covariance structure among the relatives sampled that reflect the genetic coefficients of relatedness according to the laws of heredity, and the environmental influence in common.

Because MZs share 100% of their additive effects (A) their additive genetic kinship coefficient is fixed at 1.0; DZs only share about 50% of these effects, so their coefficient is fixed at 0.5. MZs also share 100% of their dominance genetic effects (D) while DZs share less still (25%), so the coefficients for dominance are set at 1.0 for MZs and only 0.25 for DZs. Additive and dominance genetic effects are independent so can be modelled at the same time. So, for MZs G = 1A + 1D, while for DZs G = 0.5A + 0.25D.

The shared environmental factors (those that contribute to within-pair similarity, such as the rearing environment) are assumed to contribute in equal measure to the covariance of MZs and DZs, so the shared environment (C) coefficient is fixed at 1.0 for both. The non-shared environment effects (E), by definition, are uncorrelated between pairs of twins because these include aspects of the environment that contribute to twin pair differences, so these are captured under the proportion of variance that does not covary within pairs; this parameter also captures measurement error.

It is not possible to evaluate dominance effects and those of the shared environment simultaneously in the same model if twins reared together are used; this is because C and D have opposing effects on MZ and DZ correlations – while C makes DZ twins more similar to MZ twins, D makes them more dissimilar to MZ twins, compared with a scenario where the covariance between a twin pair is solely due to additive genetic effects (Heath et al., 1989). Two models can be run in order to estimate each separately – a model that estimates A, C and E, and a model that estimates A, D and E.

The most straight forward method of partitioning the variance using MZ and DZ twins is through comparison of MZ and DZ twin correlations. The more sophisticated method models the covariances among MZs and DZs and the differences between MZ and DZ covariances. First I will first demonstrate how heritability can be estimated using twin correlations. I will then explain the more sophisticated techniques.

4.5.1. Estimating heritability using twin correlations

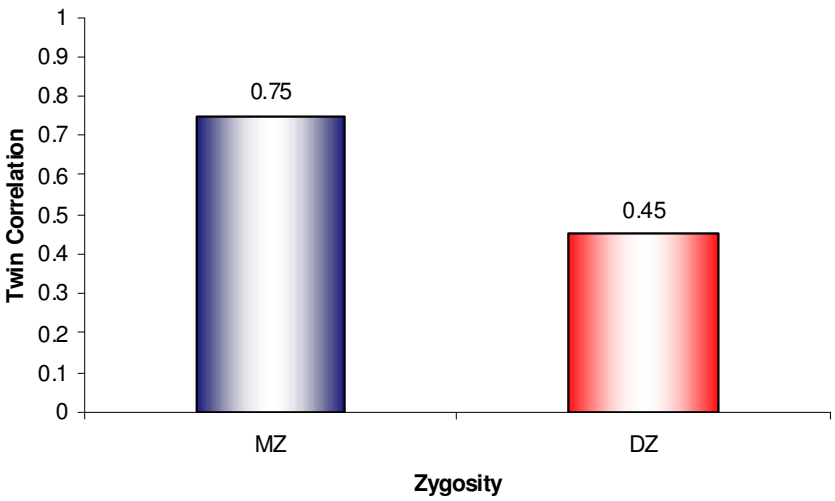
Any correlation between MZ pairs and DZ pairs could be due to common genetic effects (A and D) and shared environment effects (C), but as explained above, the common genetic effects are fewer for DZ pairs than for MZ pairs, while their shared environment effects are thought to be the same. Any difference between MZ pairs (uncorrelated variance) is due only to non-shared environmental effects and measurement error. Taking this information in to account the correlations between MZs and DZs can be used to estimate A, C and E,

and A, D and E (estimating C and D in two separate models) using a simple formula proposed by Falconer (Falconer & MacKay, 1996), explained below.

For an ACE model the additive genetic effect can be estimated by doubling the difference between the MZ and DZ resemblance, because any disparity between the MZ and DZ correlations is assumed to be due only to differences in additive genetic relatedness (a difference of 50% for additive genes): $r_{MZ} = A + C$; $r_{DZ} = (0.5 * A) + C$. So, if we know the MZ and DZ correlations it is easy to estimate A from a simple equation: $A = 2(r_{MZ} – r_{DZ})$. Because the MZ correlation is comprised only of A and C, if we know A we can easily calculate C, because $C = r_{MZ} – A$. Lastly, any difference between the MZ twins is explained by unique environment and measurement error, and is the residual variance that is uncorrelated between them: $E = 1 – r_{MZ}$. Figure 4.2 illustrates how twin correlations may be used to estimate A C and E.

For an ADE model, the same approach is followed. So we know that $r_{MZ} = A + D$, while $r_{DZ} = (0.5 * A) + (0.25 * D)$. Knowing this, we can calculate A if we know the MZ and DZ correlations, as follows: $A= (4 * r_{DZ}) – r_{MZ.}$ Just as before, because the MZ correlation is comprised only of A and D, if we know A we can estimate D by subtracting A from the MZ correlation: $D = r_{MZ} – A$. Lastly, E consists of the remaining variance after the MZ similarity has been partitioned out: $E = 1 – r_{MZ}$.

Figure 4.1. Estimating A C and E from twin correlations



ACE model

$A = 2 * (r_{MZ} – r_{DZ})$
$A = 2 * (0.75 – 0.45)$
$A = 2 * 0.30$
$A = 0.60$

$C = r_{MZ} – A$
$C = 0.75 – 0.60$
$C = 0.15$

$E = 1 – r_{MZ}$
$E = 1 – 0.75$
$E = 0.25$

4.5.2. Estimating heritability using twin covariances

Heritabilty can be modelled with much greater precision using variances and covariances between twins rather than correlations because more information is utilised – the variance as well as the covariance. The other advantage of this method over correlations is that 95% confidence intervals are provided for parameter estimates, as well as goodness-of-fit statistics for the model as a whole. For this reason, this more sophisticated technique is used in this thesis.

Maximum likelihood structural equation modelling organises the variances and covariances of each twin pair into a 2*2 matrix; matrix algebra is then used to estimate the value of the A, C (or D), and E parameters from the variance and covariance structures observed among MZs and DZs. The model starts by stipulating that the variation in the trait of interest is composed of A, C (or D) and E for every individual in the sample. An important assumption inherent is that the effects of genes and environments on each individual included in the analysis are the same, regardless of zygosity or birth order. To eliminate any potential effects of birth order the twins can be randomly allocated to 'twin 1' or 'twin 2' prior to the analysis; checks relating to zygosity effects are also important, these are discussed below in the following section ('the Saturated Model', section 4.5.2.1.).

The twin coefficients described above are used to derive expected variance-covariance matrices for MZ and DZ twins: MZs share 100% of their covariance for A, C and D, but DZs share 50% of the covariance for A, 25% for D and 100% for C. The variance-covariance matrices for the two zygosities are organised to reflect this, as follows:

MZ variance and covariance[a] (circled) matrix (adapted from Institute of Psychiatry, 2009):

	Twin 1	Twin 2
Twin 1	$\sigma^2_A + \sigma^2_C + \sigma^2_D + \sigma^2_E$	$\sigma^2_A + \sigma^2_D + \sigma^2_C$
Twin 2	$\sigma^2_A + \sigma^2_D + \sigma^2_C$	$\sigma^2_A + \sigma^2_C + \sigma^2_D + \sigma^2_E$

[a] C and D are both included for theoretical clarity although C and D would not be included in the same analysis model if twins reared together are used.

DZ variance and covariance[a] (circled) matrix (adapted from Institute of Psychiatry, 2009):

	Twin 1	Twin 2
Twin 1	$\sigma^2_A + \sigma^2_C + \sigma^2_D + \sigma^2_E$	$(0.5 * \sigma^2_A) + (0.25 * \sigma^2_D) + \sigma^2_C$
Twin 2	$(0.5 * \sigma^2_A) + (0.25 * \sigma^2_D) + \sigma^2_C$	$\sigma^2_A + \sigma^2_C + \sigma^2_D + \sigma^2_E$

[a] C and D are both included for theoretical clarity although C and D would not be included in the same model if twins reared together are used.

The variance-covariance values in the matrices for MZs and DZs will differ for different hypothetical values of A, C (or D) and E. Maximum likelihood structural equation model-fitting provides estimates for A, C (or D) and E by producing a very large number of possible parameter values and comparing them one at a time to the structures observed in the actual data set in an iterative process. The estimates finally selected are those that produce variance-covariance structures that most closely resemble the actual data (Neale & Maes, 2001; Plomin et al., 2008).

A number of goodness-of-fit statistics are produced that indicate whether the parameter estimates obtained through the maximum-likelihood process represent the observed data well. The chi-squared goodness-of-fit statistic summarises the discrepancy between the observed data values and those predicted under the specified model (e.g. ACE). The chi-squared statistic obtained for a specified genetic model (e.g. ACE) can be formally tested by comparing it to the chi-squared statistic obtained for a model that simply describes the data (a saturated model). This is explained in more detail below.

4.5.2.1. The saturated model

The saturated model makes no assumptions about the data, but fully describes the data using the maximum number of free parameters which, for 1 phenotype in the simple case, includes[14]: 4 means (twin 1 MZs; twin 2 MZs; twin 1 DZs; twin 2 DZs), 4 variances (twin 1 MZs; twin 2 MZs; twin 1 DZs; twin 2 DZs), and 2 covariances (MZs, DZs). The saturated model fulfils a number of roles: (1) it allows the researcher to test the assumptions of the univariate twin model – namely, that the variances are the same across twin 1 and twin 2, and across zygosity – if these are violated then the model may be adjusted accordingly to account for heterogeneity; (2) it allows the researcher to account for mean differences within the data if necessary – across twins, across zygosities, or across both – and; (3) it provides a baseline against which to judge the fit of subsequent alternative genetic models (e.g. ACE and ADE).

The goodness of fit of specified genetic models (e.g. ACE and ADE model) are then tested against the saturated model. Specified genetic models (e.g. ACE model) are reduced or constrained models that are 'nested' within the saturated model – that is, they contain fewer parameters than the maximum number of free parameters needed to describe the data because they reflect assumptions inherent in that particular genetic model (e.g. the standard ACE model assumes that the variances across MZs and DZs are the same). The fit of the reduced nested model can be tested against the fit of the fuller saturated model to ascertain if there is a significant drop in fit when the necessary constraints of the specified genetic model (that reflect the assumptions) are imposed on the data, using the Likelihood Ratio Test (LRT). The log likelihood (-2LL) of each model (saturated and ACE) and the degrees of freedom[15] (df) are reported; for each constrained parameter in the specified genetic model, 1 df is added. Mx uses the chi-square (χ^2) distribution to assess if the change in the -2LL, given the increase in df, represents a significant drop in fit from the saturated model by calculating a p-value[16]. A significant p-value (for a given alpha level) indicates that the imposed constraints were not reflected in the actual data (i.e. the model

[14] This is the number of free parameters in the univariate model that includes only one trait and two groups (MZs and DZs).
[15] df = *n* observed statistics – *n* estimated parameters.
[16] For a change in *df* of 1, the statistically significant change in χ^2 is 3.84 for an alpha level of 0.05.

is unlikely), and a different genetic model may be more appropriate, or the existing model requires adjustments.

4.5.2.2. Identifying the best-fitting genetic model

The pattern of the intraclass correlations indicates the nature of the genetic influence on any given trait. A large difference between MZ and DZ correlations suggests high heritability, but a DZ correlation that is substantially less than half that observed between MZs suggests the presence of genetic dominance (or other more complex effects, discussed later). Such an observation would warrant an ADE model to be fit as well as the standard ACE model. In order to choose between them, both can be fit and tested against the saturated model using the LRT.

ACE and ADE models are independent (non-nested) because they specify different parameters. So if both models fit the data well (no significant decrease in fit compared with the saturated model) they cannot be tested against one another using the LRT. This is the case with any non-nested competing genetic models. Other statistics are available to help choose the best-fitting model from independent models – Aikaike's Information Criterion (AIC)[17] and the Bayesian Information Criterion (BIC)[18]. Both statistics penalize models for increasing complexity, and/ or take account of sample size (Burnham & Anderson, 2002; Raftery, 1995). A caveat of the LRT is that the likelihood of the model increases with additional parameters – so a greater number of free parameters will improve the goodness-of-fit of the model without regard for the number of parameters that actually explain the data (e.g. if a data set is entirely explained by A and E alone, adding a C component will improve the model fit even if it contributes nothing to the data). AIC and BIC both penalize models for the number of parameters to be estimated, and in each case lower values indicate a better-fitting model.

BIC increases with unexplained variation in the observed data and the number of parameters in the model, and also takes sample size in to account by increasing the

[17] AIC=χ^2 -2*df*.
[18] BIC = χ^2 – (*df* x ln(n)), where n is the total number of pairs.

penalty as the sample size gets larger. Lower values imply either fewer parameters, a better-fitting model given the observed data, or both. AIC also rewards goodness-of-fit while penalizing for model complexity (parameter number), and favours the model that explains the greatest proportion of the observed data with the fewest parameters. Specifically, AIC makes the researcher pay a penalty of two for every parameter that is estimated. Although the two statistics are very closely related, in general BIC places a higher value on parsimony than does AIC and so penalizes additional parameters more strongly, and it takes sample size into account.

BIC and AIC do not provide statistical tests of significance to evaluate differences between models, rather they aid in model selection. Better-fitting models are indicated by increasingly negative values. The absolute values of the respective statistics have no meaning in themselves, and are only useful for estimating relative differences between models. As a general rule the lowest (most negative) AIC or BIC value is to be preferred but guidelines have been published regarding the magnitude of the AIC and BIC differences between models. Burnham and Anderson (Burnham & Anderson, 2002) suggest that an AIC difference of < 2 does not provide much support for one model over another; an AIC difference of 4-7 suggests considerably less support for the model with the higher AIC, and a difference of > 10 indicates that the model with the highest AIC receives no support. Raftery (Raftery, 1995) has provided BIC difference values that correspond to varying levels of evidence: 0-2 = ‘weak’; 2-6 = ‘positive’; 6-10 = ‘strong’; >10 = ‘very strong’ (Raftery, 1995).

BIC and AIC can only be used to compare models that utilise the same set of data, such as non-nested models that represent the same dataset using different constraints. AIC and BIC can also be used to aid in model selection from nested models, along with the LRT. So, for nested models there are three goodness-of-fit statistics available. Of course, one hopes that in any analysis they all agree with one another but this is not always the case, and so the researcher must decide which statistic to follow. A consideration is that AIC and the LRT become less reliable as the sample size increases, and both tend to prefer the model with more parameters (Mulaik et al., 1989); the LRT, in particular, is sensitive to small and relatively trivial differences between models when samples are large, as are all

statistical tests of significance (Mulaik et al., 1989). Markon and Krueger (Markon & Krueger, 2004) used data simulations to test the robustness of a number of goodness-of-fit statistics and concluded that BIC was preferable to AIC, especially with large samples and complex models (those with many parameters). The choice of statistic therefore benefits from a consideration of the sample size and the number of parameters in the model; and it is always advisable to observe the parameter estimates and use the 95% confidence intervals to aid in determining which parameters are contributing importantly.

4.5.2.3 The principle of parsimony

The scientific principle of parsimony (Occam's Razor) recommends that the simplest model that can sufficiently explain the data with the fewest number of parameters should be preferred. Following this line of reasoning, simplified sub-models that are nested within the full ACE or ADE model, but with fewer variance components, are subsequently tested against the full model using the LRT. A model that drops only the additive genetic component of variance (CE) is run; then a model is tested that drops the shared environmental effects (or genetic dominance) (AE); finally, a model can be tested that retains only the unique environment parameter (E model). The E variance component is always retained because this parameter captures random measurement error and it is not empirically sound to assume that any phenotype can be measured without error.

4.5.2.4. Path analysis

The equations that are used to derive the predicted variance and covariance matrices can also be expressed using path diagrams which provide a schematic representation of the relationships under the specified model (e.g. ACE or ADE). Path analysis expresses variances and covariances as regression coefficients and correlations, but mathematically they are equivalent. Standard symbols are used to represent the latent and observed variables and relationships between them, illustrated below (Institute of Psychiatry, 2009):

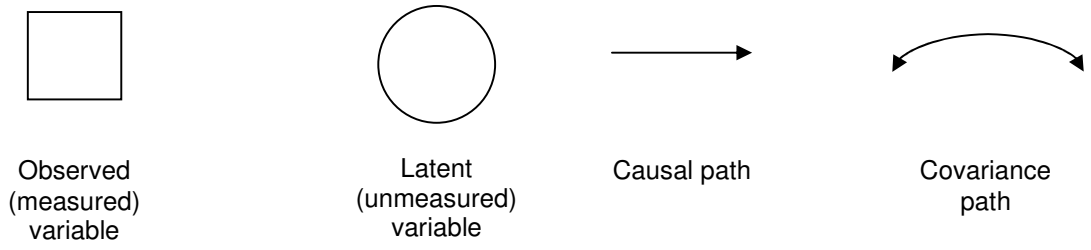


Figure 4.3 illustrates the univariate ACDE model represented as a path diagram (a model including C and D is shown for theoretical purposes only, but as discussed earlier a model would not include both C and D if only twins reared together are sampled). The rectangular boxes labelled “Twin 1” and “Twin 2” denote the measured phenotypes (e.g. appetitive traits) and the circles represent latent effects on the phenotype including additive genetic effects (A), dominance genetic effects (D), shared-environment effects (C) and non-shared (E) environment effects. The single-headed straight arrows indicate the independent statistical effect of each latent variable on the phenotype in the form of partial regression coefficients. The latent variables are allowed to covary between Twin 1 and Twin 2 as shown by the curved double-headed arrows – additive genetic covariance is fixed at 1.0 for MZ twins and 0.5 for DZ twins, genetic dominance covariance is fixed at 1.0 for MZ twins and 0.25 for DZ twins, and the shared environment effect is fixed at 1.0 for both – the non-shared environment effect (and measurement error) does not covary between twins. The curved double-headed arrow loops on each latent variable represent the variance of each variable (which equates to the covariance of the variable with itself), and in each case the variance is standardised to be 1.0.

Wright, who developed path analysis (Wright, 1921), postulated that the covariance between any two variables can be calculated by summing all ‘legitimate chains’ that connect the two variables, and the mathematical value of a chain is the product of all its constituent path coefficients. He set out rules about how paths may be traced (Wright, 1934):

1. An arrow can be traced backward and then forward along the next path, or forward from one variable to another, but not forward and then back.
2. A variable can only be traced through once in any chain of paths.

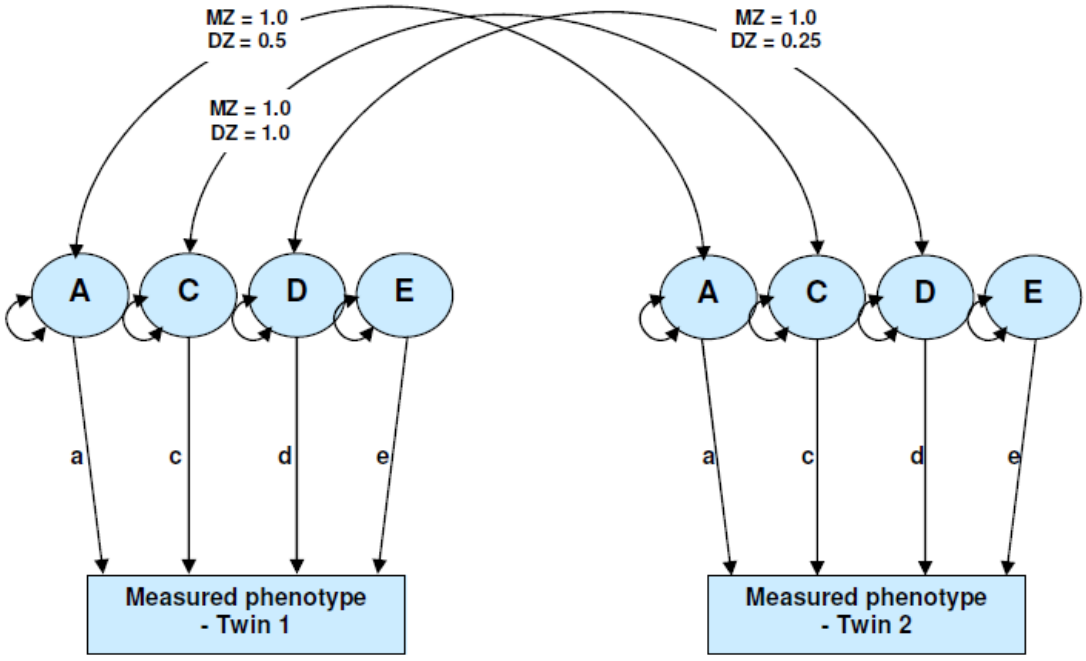
3. No more than one double-headed arrow can be included in each path-chain; so, the looped arrows from each latent variable to itself is only included if the chain does not include another double-headed arrow such as a correlation path.

Following these rules, in Figure 4.3 the covariance between MZs for the two measured phenotypes = (a * 1.0 * a) + (c * 1.0 * c) + (d * 1.0 * d), which = (1.0 * a^2) + (1.0 * c^2) + (1.0 * d^2), or $a^2 + c^2 + d^2$; for DZs the covariance for the two measured phenotypes = (a * 0.5 * a) + (c * 1.0 * c) + (d * 0.25 * d), which = (0.5 * a^2) + (1.0 * c^2) + (0.25 * d^2), or $0.5a^2 + c^2 + 0.25d^2$. And for each twin, the variance of their measured phenotype is: (a * 1.0 * a) + (c * 1.0 * c) + (d * 1.0 * d) + (e * 1.0 * e), which = $a^2 + c^2 + d^2 + e^2$ (Plomin et al., 2008).

4.5.2.5. Data preparation

Analyses for heritability are conducted on data that have been corrected for the sample effects of age and sex by creating residual scores. This is because the age and sex of twins is exactly correlated within each same-sex pair so variation within age and sex at the point in time that the phenotype was measured can contribute to twin similarity and inflate the shared environment effect (Mcgue & Bouchard, 1984); this would also result in a lower correlation between opposite DZ pairs who do not share their sex, compared with the same-sex DZ pairs. In addition, Maximum Likelihood modelling requires that the data be normally distributed. As mentioned earlier, in order to remove any effect of birth order the twins are randomly allocated to ‘Twin 1’ and ‘Twin 2’ groups for the analyses.

Figure 4.2. Path diagram of the ACDE model for one phenotype



Univariate path diagram. The circles indicate latent influences on the measured phenotype for each twin which include additive genetic effects (A), shared environmental effects (C), dominance genetic effects (D), and unique environmental effects and error of measurement (E). The rectangular boxes represent the measured phenotype for each twin. The straight single-headed arrows show the causal paths (i.e. the phenotype for each twin results from the collective influence of A, C, D and E). The curved double-headed arrows show the covariance paths between the twins. A and D are perfectly correlated between MZ pairs so the coefficient is fixed at 1.0; for DZ twins the coefficients of relatedness are set at 0.5 for A and 0.25 for D. The coefficient for the shared environment effect is fixed at 1.0 for MZs and DZs as this is assumed to be the same regardless of zygosity. Unique environmental influences are responsible for twin differences, so E is uncorrelated between twins. The proportion of variance explained in the measured phenotype by the latent factors may be estimated by squaring the path coefficients: (e.g. a^2, c^2, d^2, e^2).

4.6. Estimating genetic and environmental effects for multiple phenotypes using twins

The univariate model can be extended to include multiple phenotypes or multiple time-points. Multivariate genetic model-fitting provides a means for testing if there are common genetic, shared environmental or unique environmental influences underlying two or more different traits, or underlying the same trait at different time points. It also provides information about the relative importance of the underlying influences that account for phenotypic correlations – e.g. is the correlation between 'food responsiveness' and 'enjoyment of food' due to genes that influence both traits, or environments that encourage both traits?

Multivariate genetic model-fitting uses the same principles as univariate model-fitting, except that the within-pair cross-covariation between different traits or time-points is the primary focus rather than within-pair within-trait covariation – e.g. is Twin 1's appetite associated with Twin 2's weight? As with univariate analyses, multivariate heritability may be estimated using within-pair correlations or covariance between the twins. There are a number of different theoretical representations for multivariate data, which provide different types of information about the genetic relatedness, and make different assumptions about the underlying common and unique causal pathways. The model that offers the best representation of the data may be selected using the AIC and BIC criterion explained earlier. These models are explained in more detail below. For ease of explanation in each case I will describe the models simply using A, C and E. However, all of the models may be run substituting D for C.

4.6.1. Cross-twin cross-trait correlations

Cross-twin cross-trait correlations form the basis of the multivariate approach; they indicate how one twin's scores for one trait (or time-point) vary in comparison to the other twin's scores for the other trait (or time-point). The same equations that are used to estimate univariate heritability from cross-twin within-trait correlations may be used to estimate multivariate heritability from cross-twin cross-trait correlations. The resulting estimates of A, C and E indicate the extent to which common genetic, shared environment

or unique environment effects explain phenotypic associations between different traits. The same theoretical inferences are applied for the cross-correlations – if common genetic factors are driving the association between two traits the MZ cross-twin cross-trait correlation will be higher than the DZ correlation. Calculating the difference between them and doubling it will indicate the proportion of the phenotypic correlation accounted for by common genetic factors; subtracting the cross-trait heritability from the MZ phenotypic correlation gives an estimate of the proportion explained by common shared environment effects, and the remaining chunk of the phenotypic correlation is explained by unique environment effects (this does not include error because it is correlated within each individual).

Certain patterns in the correlations indicate the nature of the shared influences. Significant correlations across traits at the level of the individual (e.g. significant Pearson's correlations) are the starting point and these suggest common influences are at play. Significant cross-twin cross-trait correlations indicate a familial influence that can be coming from either common shared environment effects or common genes. A large difference between the MZ and DZ cross-correlations suggest common genetic influences are more important, while very little difference between MZ and DZ cross-correlations would imply that it is mainly common shared environment effects. If significant within-individual cross-trait correlations are found but cross-twin cross-trait correlations are not significant, it is likely that common unique environmental factors are driving the phenotypic correlations. The phenotypic correlations set a ceiling for the MZ correlations so if the difference between the MZ cross-trait correlation and the phenotypic correlation is small, common influences of the non-shared environment are not contributing importantly to the covariation between the two traits.

4.6.2. Estimating multivariate heritability using twin covariances

The univariate covariance modelling techniques may also be extended to estimate the shared genetic and environmental influences on multiple traits. The essence of the method is analogous, except that twin covariation across different traits is modeled rather than twin covariation across the same trait. In the case of two measured phenotypes, two models

are most commonly used – the Cholesky Decomposition Model and the Correlated Factors Model, which is a mathematically transformed version of the former, both described below.

4.6.2.1. Cholesky Decomposition Model

The Cholesky Decomposition Model (Figure 4.4) specifies as many sets of latent factors as there are measured phenotypes, so for two phenotypes there will be two sets of A, C and E. The order in which the observed measures are entered into the model impacts upon the organization of the covariance because the first set of latent factors (A1, C1 and E1) explain variance in the first variable as well as covariance between the first and second variable. The second set of latent factors (A2, C2 and E2) only explain residual variance in the second variable not explained by A1, C1 and E1, but they do not explain variance in the first variable.

The same coefficients for MZ and DZ covariances that are used in the univariate model are implemented across traits in the multivariate case (i.e. the coefficient of additive genetic relatedness is 1.0 for MZs and 0.5 for DZs, for dominance genetic effects it is 1.0 for MZs and 0.25 for DZs, the coefficient for the effects of the shared environment is 1.0 for both, and non-shared environment effects (and random measurement error) are uncorrelated between pairs). The variances and covariances are calculated using the same path rules as those described for the univariate model (see Figure 4.4 for a full explanation).

4.6.2.2. Correlated Factors Model

If the causal direction of the variables is unknown, the Cholesky Decomposition Model can be transformed into a Correlated Factors Model (Loehlin, 1996) which provides information about the univariate and the shared influences on the variables without giving priority to one variable over another. The path diagram of this model is shown in Figure 4.5. The following parameters are equivalent in the Correlated Factors Model and the Cholesky

Decomposition Model: $x_1 = a_1$; $x_2 = \sqrt{(a_{12}^2 + a_2^2)}$; $y_1 = c_1$; $y_2 = \sqrt{(c_{12}^2 + c_2^2)}$; $z_1 = e_1$; $z_2 = \sqrt{(e_{12}^2 + e_2^2)}$ (Loehlin, 1996).

The Correlated Factors Model provides two pieces of information about the shared latent influences on the measured phenotypes. The aetiological correlations (genetic [r_A], shared environmental [r_C], and non-shared environmental [r_E]) indicate the extent to which the same genetic or environmental influences influence the two measures[19]. As with any correlation a high value implies that most of the latent influences across the two traits are the same. An important point is that the aetiological correlations are independent of each univariate heritability; so it is possible to observe very high additive genetic correlations with very low heritability estimates for each trait. This would occur if genetic influences did not play a very important role for either characteristic, but the few genes that are involved influence both characteristics.

The other useful information provided are 'bivariate' estimates of heritability, the shared environment and the non-shared environment. These quantify how much of the phenotypic association is explained by common genetic, shared environment or unique environmental influences on both traits, and the sum of the three bivariate esimtates equals the phenotypic correlation. They are calculated by tracing the paths that contribute to phenotypic covariation between the traits[20]. While the aetiological correlations indicate the extent to which the genetic factors or environments that influence each characteristic are the same, the bivariate estimates highlight which common factors (genetic or environmental) are more important in driving the observed phenotypic association. Bivariate estimates are also independent of the genetic correlation – for example, a very high genetic correlation might be observed for two traits that are not particularly heritable

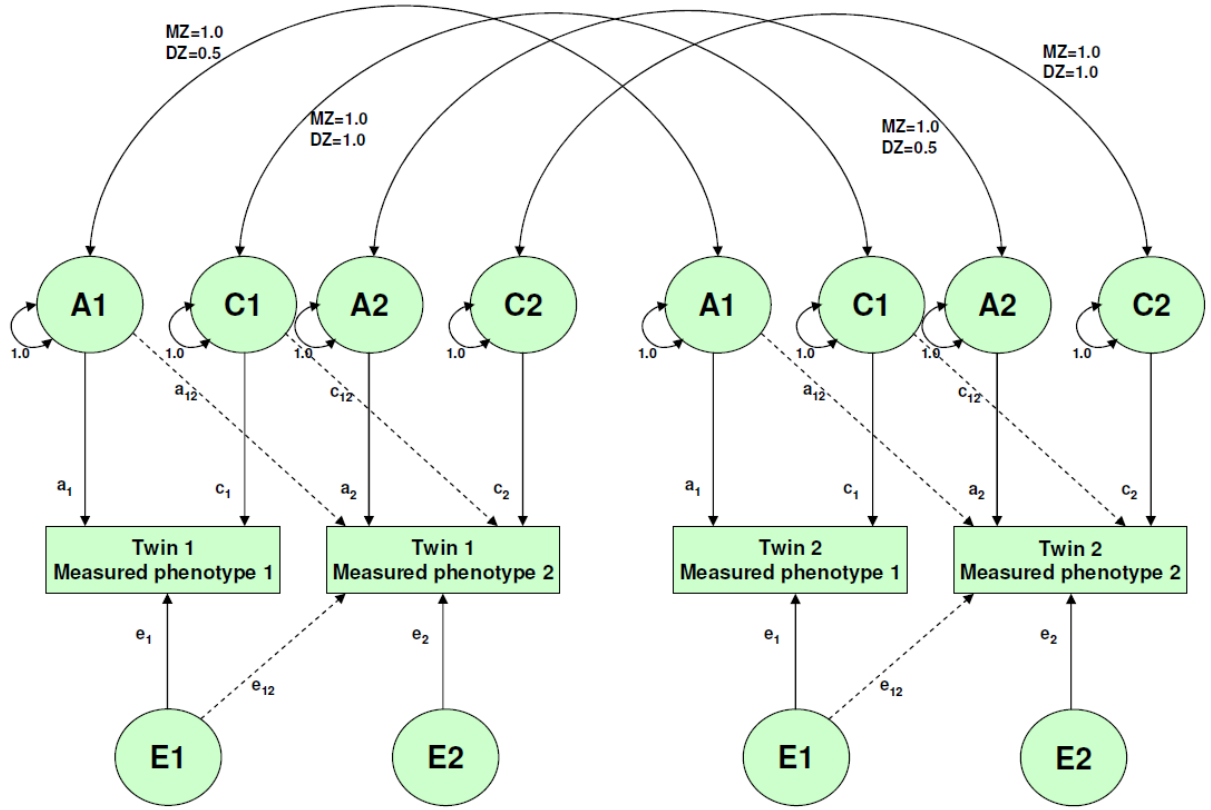
[19] The aetiological correlations are calculated simply from the covariation paths and the univariate components of variance. For example, the additive genetic correlation is calculated by dividing the additive genetic covariation path coefficient between Phenotype 1 and Phenotype 2 (a_{12}) by the square root of the total univariate heritability on Phenotype 2: $a_{12} / \sqrt{(a_{12}^2 + a_2^2)}$, or a_{12} / x_2. An important point is that the value of the correlation is the same regardless of the variable that is assigned as Phenotype 1 or Phenotype 2. The shared environment and unique environment correlations are calculated in the same way using the equivalent paths (Loehlin, 1996).

[20] The bivariate heritability is calculated easily from the Correlated Factors Model by multiplying the two path coefficients for the univariate heritabilities by the genetic correlation: $x_1 * r_A * x_2$ (Plomin et al., 2008); the bivariate shared environmental effect and bivariate non-shared environmental effect are calculated in the same way using the equivalent paths. The bivariate estimates can then be converted to percentages by dividing each by the phenotypic correlation and multiplying by 100 to aid interpretation.

which makes it unlikely that the common genetic influences are playing an important role in driving phenotypic correlation, making the bivariate heritability low.

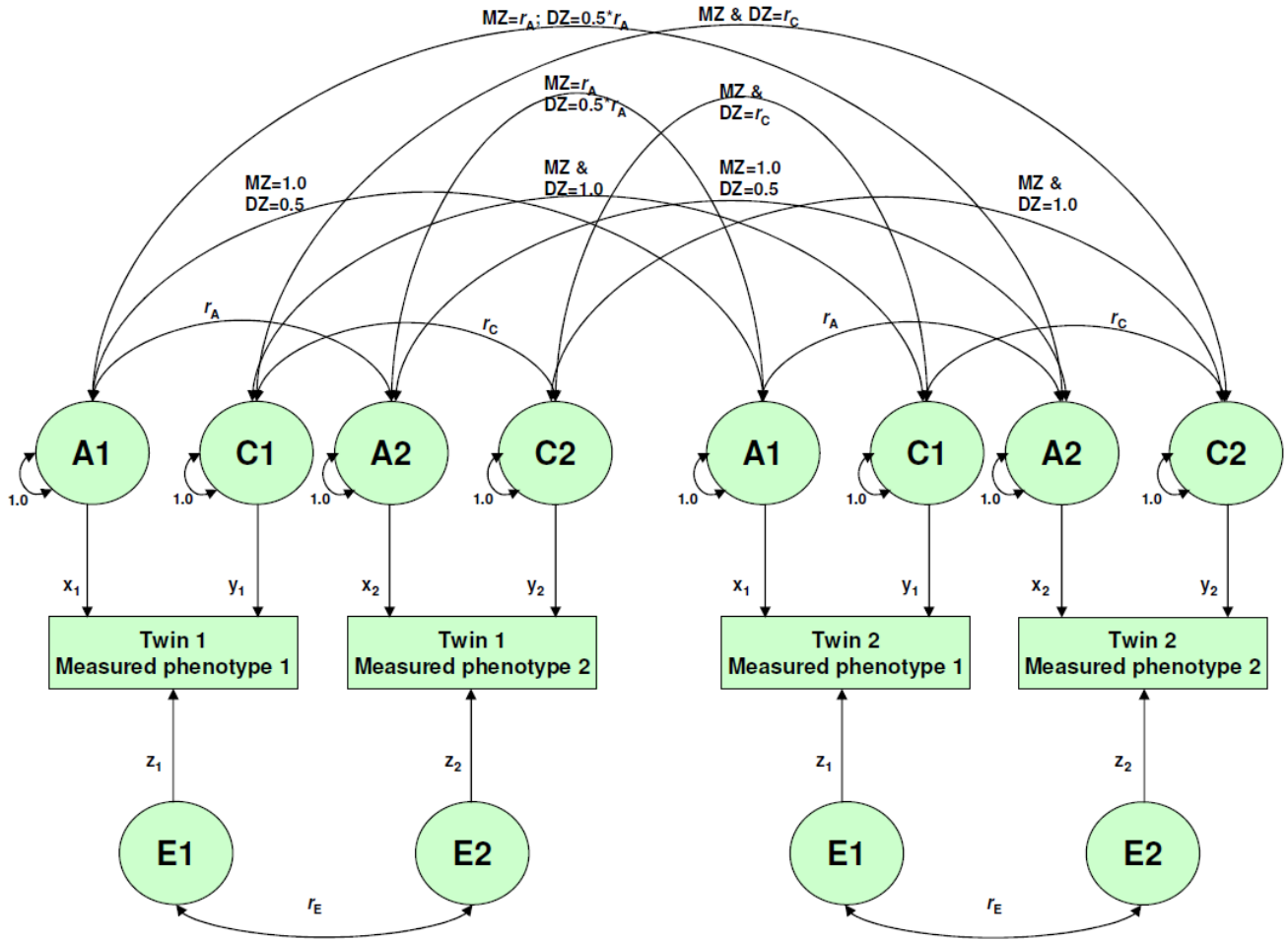
The Cholesky Decomposition Model and the Correlated Factors Model can be used to estimate shared influences on more than two traits. In addition, two other models are commonly used for multivariate analyses that include more than two phenotypes – the Independent Pathway Model and the Common Pathway Model, both explained below.

Figure 4.3. Bivariate ACE Cholesky Decomposition Model for a twin pair



Cholesky Decomposition Model for two twins. For each twin the variance for Phenotype 1 = $a_1^2 + c_1^2 + e_1^2$; the variance for Phenotype 2 consists of variance in common with Phenotype 1 (dotted paths) as well as variance specific to Phenotype 2 (independent of Phenotype 1), which = $a_{12}^2 + c_{12}^2 + e_{12}^2 + {}^2a_2^2 + c_2^2 + e_2^2$. The total additive genetic effect on Phenotype 1 is: (a_1 * 1.0 * a_1), which = a_1^2; the total additive genetic effect on Phenotype 2 is: (a_{12} * 1.0 * a_{12}) + (a_2 * 1.0 * a_2), which = $a_{12}^2 + a_2^2$. The total shared environmental effects on each phenotype are calculated in the same way using the c_1 c_{12} and c_2 paths, and the unique environments effects using the e_1 e_{12} e_2 paths. The additive genetic effect on Phenotype 2 can be broken down into that which is shared with Phenotype 1 and that which is specific to Phenotype 2 (independent of Phenotype 1): additive genetic effects on Phenotype 2 that are shared completely with Phenotype 1 = (a_{12} * 1.0 * a_{21}), or a_{21}^2; additive genetic effects on Phenotype 2 that are independent of those on Phenotype 1 = (a_2 * 1.0 * a_2), or a_2^2. The shared environment effects and unique environment effects on Phenotype 2 that are in common with Phenotype 1 or independent can be broken down and calculated using the same method. The total covariation between MZs for the two phenotypes is: (a_1 * 1.0 * a_{12}) + (c_1 * 1.0 * c_{12}), which = $a_1a_{12} + c_1c_{12}$. The total covariation between DZs for the two phenotypes is: (a_1 * 0.5 * a_{12}) + (c_1 * 1.0 * c_{12}), which = $0.5(a_1a_{12}) + c_1c_{12}$. The additive genetic covariation for MZs is: (a_{11} * 1.0 * a_{12}), which = a_1a_{12}; for DZs it is: (a_{11} * 0.5 * a_{12}), which = 0.5 a_1a_{12}. The shared environment covariation between MZs and DZs is calculated in the same way using the c_1 and c_{12} paths but fixing the coefficient of relatedness at 1.0 for MZs and DZs.

Figure 4.4. Bivariate ACE Correlated Factors Model for a twin pair



Correlated Factors Model for two twins. For each twin the variance for Phenotype 1 = $x_1^2 + y_1^2 + z_1^2$; the variance for Phenotype 2 = $x_2^2 + y_2^2 + z_2^2$. The total additive genetic effect on Phenotype 1 is: (x_1 * 1.0 * x_1), which = x_1^2; the total additive genetic effect on Phenotype 2 is: (x_2 * 1.0 * x_2), which = x_2^2. The total shared environmental effects on each phenotype are calculated in the same way using the y_1 and y_2 paths, and the unique environments effects using the z_1 and z_2 paths. The genetic correlation (r_A) is calculated from the Cholesky Decomposition Model (see Figure 4.4) using the following equation: $a_{12} / \sqrt{(a_{12}^2 + a_2^2)}$; the shared environment correlation (r_C) and unique environment correlation (r_C) are calculated in the same way using the equivalent paths. The bivariate heritability (contribution of common genes to the phenotypic correlation) is: $x_1 * r_A * x_2$; the bivariate shared environmental effect is $y_1 * r_C * y_2$; the bivariate unique environmental effect is $z_1 * r_C * z_2$; the sum of the bivariate estimates is equal to the phenotypic correlation.

4.6.2.3. Independent Pathway Model

The Independent Pathway Model makes slightly different assumptions about the underlying nature of the phenotypes to the Cholesky and Correlated Factors Models. If four phenotypes are included in the model the Cholesky (or Correlated Factors) Model may be thought of as a saturated model, in that it estimates the maximum number of variances and covariances among phenotypes. On the other hand, when four phenotypes are examined, the Independent Pathway Model is more constrained because the theoretically derived structure imposed on the data has fewer parameters (24 instead of 30). Because the Independent Pathway Model is nested within the fuller Correlated Factors Model in the four phenotype case it can be formally tested against it by means of the LRT; if it does not provide a significantly worse fit compared to the data it offers a more parsimonious representation of the data than does the Correlated Factors Model. If only three phenotypes are included the Correlated Factors Model and Independent Pathway Model are equivalent, in that they estimate the same number of parameters. Figure 4.6 illustrates the Independent Pathway Model as a path diagram, but only includes three phenotypes for ease of interpretation.

The essence of the model is that the shared variance between the phenotypes is partitioned in to common genetic influences (Ac), common shared environmental influences (Cc) and common unique environmental influences (Ec), while the remaining residual variance that is specific to each phenotype is partitioned into specific A, C and E estimates for each variable[21]. So, while the Correlated Factors Model provides information about each pairwise genetic correlation, the Independent Pathway Model indicates the extent to which common pathways (e.g. Ac, Cc and Ec) influence all of the phenotypes simultaneously. An assumption of the Independent Pathway Model is that common influences act independently of one another, rather than conspire together to influence a trait.

[21] The same coefficients of relatedness between MZ and DZ twins are in place in both the Independent Pathway and Common Pathway models as have been shown in the other models.

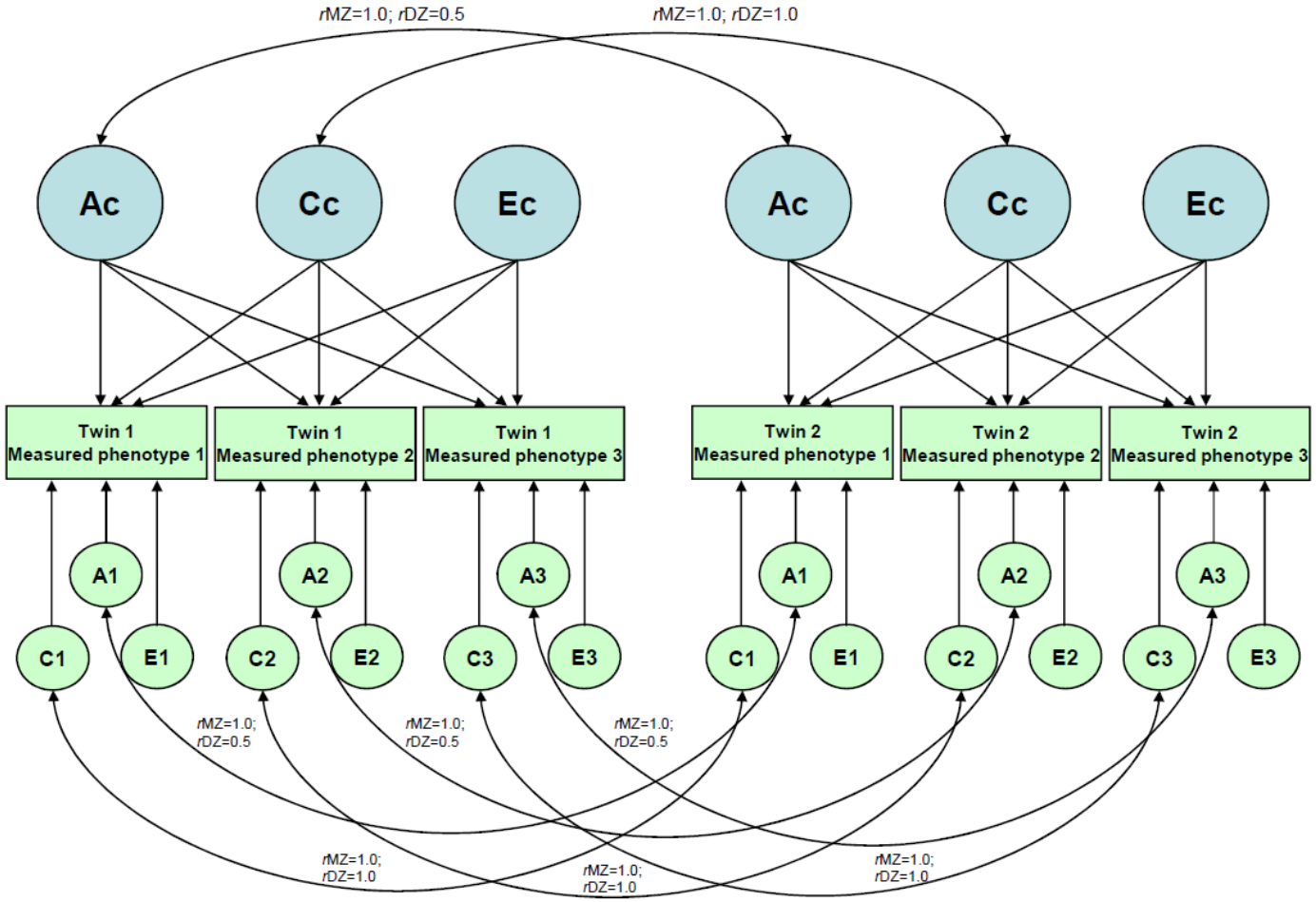
4.6.2.4. Common Pathway Model

The Common Pathway Model is even more constrained in comparison to the Independent Pathway Model, because even fewer parameters are estimated (this is also the case with only three phenotypes). It assumes that all of the common genetic and environmental variance (Ac, Cc and Ec) is mediated through a latent psychometric factor that explains variance in all the measured phenotypes. So, covariation among the phenotypes is assumed to be due to the effects of the intermediate latent phenotype. The latent factor is, in turn, influenced by additive genetic effects, shared environmental effects, and unique environmental effects common to the three phenotypes by virtue of the latent factor (Ac, Cc and Ec). There is also residual variance for each phenotype that is again partitioned into specific influences including additive genetic effects, shared environmental effects and unique environmental effects. The model is shown graphically in Figure 4.7. A key distinction between the Common Pathway Model and the Independent Pathway Model is that the common influences do not act independently of one another but in concert through the common latent factor. As the Common Pathway Model in essence is a more constrained version of the Independent Pathway Model and the Correlated Factors Model (in that there are fewer parameters) its fit can be tested against theirs using the LRT. This model provides the most parsimonious representation of the data.

4.6.3. The advantages of taking a multivariate approach

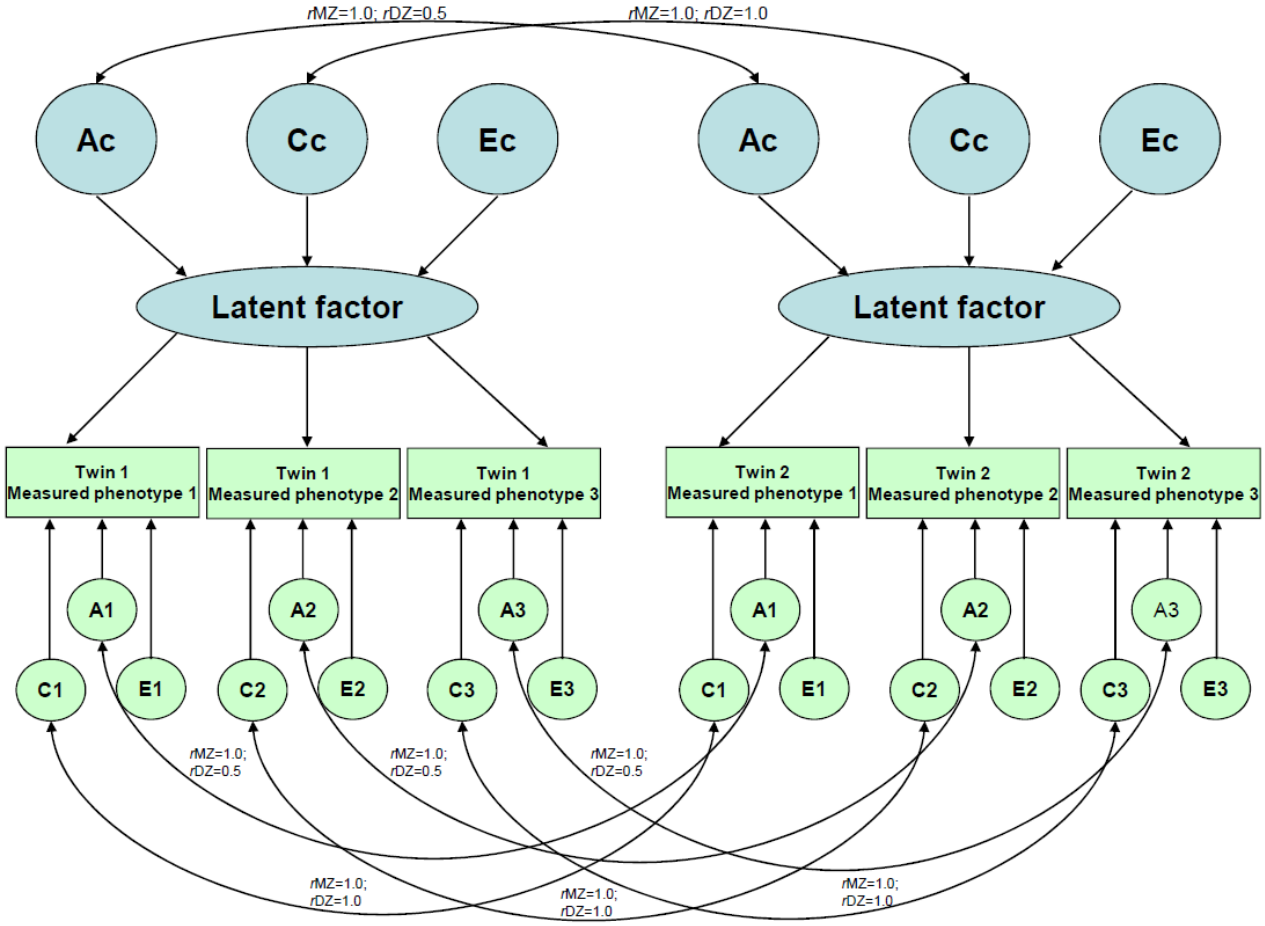
Multivariate analyses provide a richer and more complex account of the genetic and environmental causal pathways among multiple phenotypes than separate univariate models because the multivariate approach accounts for relationships within twin pairs as well as relationships between the phenotypes. Additionally, the multivariate approach has increased statistical power compared to univariate analyses when variables included in the analysis are correlated (power is discussed in more detail in section 4.8). The only consideration is that multivariate genetic analysis is extremely computer-intensive and estimating models fully (using bootstrapping techniques to provide 95% confidence intervals for all of the parameters) with three or more variables in large datasets can take several hours or even days.

Figure 4.5. Independent Pathway Model for a twin pair



Independent Pathway Model for two twins and three measured phenotypes. Each phenotype is influenced by latent additive genetic, shared environmental and unique environmental factors that are common to all three phenotypes (Ac, Cc and Ec, respectively), as well as latent additive genetic, shared environmental and unique environmental latent factors that are specific to each phenotype (A1,A2,A3,C1,C2,C3,E1,E2,E3). For each twin the total variance for each phenotype consists of all the latent influences on that trait, e.g. for Phenotype 1 the total variance = Ac + Cc + Ec + A1 + C1 + E1; the total genetic variance on Phenotype 1 = Ac + A1; the total shared environmental variance on Phenotype 1 = Cc + C1; the total unique environmental variance on Phenotype 1 = Ec + E1. The same methods are used to calculate the components of variance for the other phenotypes.

Figure 4.6. Common Pathway Model for a twin pair



Common Pathway Model for two twins and three measured phenotypes. Each phenotype is influenced by additive genetic, shared environmental and unique environmental factors that are common to the three phenotypes (Ac, Cc and Ec, respectively), but these common influences are mediated through a common latent factor that directly influences each phenotype. The three phenotypes are also influenced by latent additive genetic, shared environmental and unique environmental latent factors that are specific to each phenotype (A1,A2,A3,C1,C2,C3,E1,E2,E3). For one twin the total variance for each phenotype consists of the common latent factor influence on that trait and the specific influences on that trait, e.g. for Phenotype 1 the total variance = Latent Factor + A1 + C1 + E1. The total additive genetic variance on Phenotype 1 is calculated using path tracing rules: (Ac * Latent Factor) + A1. The total shared environmental and unique environmental influences on Phenotype 1 are calculated using the same rules. The same methods are used to calculate the components of variance for the other phenotypes.

4.7. Additional complexities

A number of factors can complicate heritability analyses, and some of these relate more specifically to the analysis of twin data. Complications arise when the assumptions of the models are violated, such as the additive genetic relatedness of DZs being higher than 50%, or the shared environment not being equal for MZs and DZs. Furthermore, interactions are possible (between siblings, between genes and the environment, and between genes themselves), and heritability may differ by sub-group. Some of these factors may be tested for, or taken in to account in the model-fitting, but others are more problematic. The main points for consideration are discussed below.

4.7.1 Assortative mating

'Assortative mating' is non-random mating (Plomin et al., 2008) and refers to individuals breeding with mates who are fairly similar on certain traits. For example, there is a spousal correlation of about 0.1-0.2 for weight and BMI (Allison et al., 1996; Mascie-Taylor, 1987; Silventoinen et al., 2003; Spuhler, 1968; Tambs et al., 1991). This phenomenon is known to influence additive genetic variance in a population because children will express the average parental phenotype. For example, if an overweight parent and a very lean parent procreate, the offspring are likely to be of average weight; on the other hand, if two overweight parents produce off-spring, their children will be heaver than average, while children of two very lean parents will be leaner than average.

Assortative mating can increase genetic variability quite substantially in a population because its effects are compounded across generations, and it has been suggested that this process could be contributing to the increase in the prevalence of obesity in the Western world (Kearsey & Pooni, 1996). This phenomenon also influences estimates of heritability derived from family studies – it inflates correlations between parents and offspring which inflates estimates of heritability derived from this method. However, estimates of heritability derived from twins are decreased, because the correlation between fraternal twins is higher (as a result of genetic similarity between fraternal twins being greater than 50%), while the correlation between identical twins remains the same

(because the genetic relatedness of identical twins remains unaffected at 100%), which results in a smaller difference between MZs and DZs and a lower estimate of heritability; instead, estimates of the shared environment are increased.

4.7.2. Equal environments assumption

Inherent in the twin method is the assumption that environmentally caused similarity between fraternal and identical twins is the same, i.e. influences such as parental treatment, teacher treatment, schooling, friends, etc is assumed to be similar for MZs and DZs. Violation of this assumption can happen if MZ twins do in fact share more similar environments than DZ twins, and the difference in environmental similarity causes the elevated covariation between the MZs compared to the DZs. If this occurs estimates of heritability are inflated because the MZ correlation will be greater, creating a larger discrepancy between MZ and DZ correlations, not caused entirely by genetic differences. On the other hand, if MZs experience a less similar environment than DZs, the reverse is true – estimates of the shared environment will be inflated because the MZ correlation is decreased, making MZ and DZ likeness more similar.

It is not possible to adjust for unequal environments within the twin model as this is an inherent assumption of the standard ACE model (insofar as the C parameter is fixed at 1.0 for both MZs and DZs). However, the question of whether MZs share more or less similar environments than DZs is an empirical one, therefore it can be, and has been, tested using a variety of different methods. A useful design to explore this issue is to compare MZ twins who have been mistakenly brought up as DZ twins (or vice versa), or pairs of twins who have misclassified themselves.

4.7.3. Sibling interaction effects and parental rating biases

It is possible that one sibling's phenotype directly influences the behaviour of his or her co-twin, which is termed a 'sibling interaction effect'. In this way, the genes that directly influence the phenotype in one twin also exert an indirect effect on the phenotype of his or

her co-twin (Eaves, 1976); this gives rise to covariation between the twins that does not result from shared causation, rather the phenotype of one twin is directly causing aspects of the phenotype in their co-twin and creating additional covariation between the pair, independent of genetic effects and the shared environment. Two such effects are possible – likeness between the twins can either be increased ('cooperation effects') or decreased ('competition effects'). For example, if one child is a rapid eater, the other child may increase his or her eating speed accordingly in order to secure 'seconds' at mealtimes (this would be a 'cooperation' effect).

Parental rating biases mimic sibling interaction effects and, likewise, there are two kinds. Contrast effects occur when raters overestimate differences between twins; MZs are much less prone to rater contrasts for genetically determined traits because they will be more similar to start with. Assimilation effects exist when raters accentuate similarities between twins; DZs are much less prone to assimilation effects for genetically determined traits because they will be less similar to start with. Because contrast effects operate more strongly for DZs their correlation is much lower than it should be, and contrast effects result in greater variance across DZs than MZs; on the other hand, assimilation effects operate more strongly for MZs so their correlation is far higher than it actually should be, and variance is increased for MZs, but not for DZs. The net result in both cases is that DZ correlations are much less than half of the MZ correlations. Exactly the same patterns are seen in the data with 'competition' and 'cooperation' effects. If patterns in the data indicate that sibling effects or parental rating effects are present (i.e. DZ correlations that are much less than half the MZ correlations, and significant differences in the variances of MZs and DZs), the model can be adjusted to account for these (Eaves, 1976; Neale & Maes, 2001; Rietveld et al., 2003; Saudino et al., 2000).

4.7.4. Heterogeneity and heritability

Heritability estimates are population- and time-specific in that they describe the relative influence of genes and environment to observed phenotypic differences (variance) in the particular population that has been sampled, at a given time point. Different populations may show different influences, as could also be the case if the same population were

measured at different times. This is an important point because if there are substantial genetic influences within one group but not in another, the true genetic effect sizes for the different groups will be masked and the overall estimate will reflect the average (Plomin et al., 2008) – e.g. heritability may be estimated at 50% for an imaginary trait called 'syndrome male', even though the trait is 100% heritable for males, and not at all heritable for females, because it is completely heritable in 50% of the sample (all of the males). This may be the case with any type of group difference. Group differences can also be incorporated into quantitative genetic model-fitting. For example, a model that allows A, C and E to differ for males and females (or other groups) can be tested against a model that equates A, C and E for males and females to see if it provides a better fit to the data. The sex-limitation model is described in more detail below.

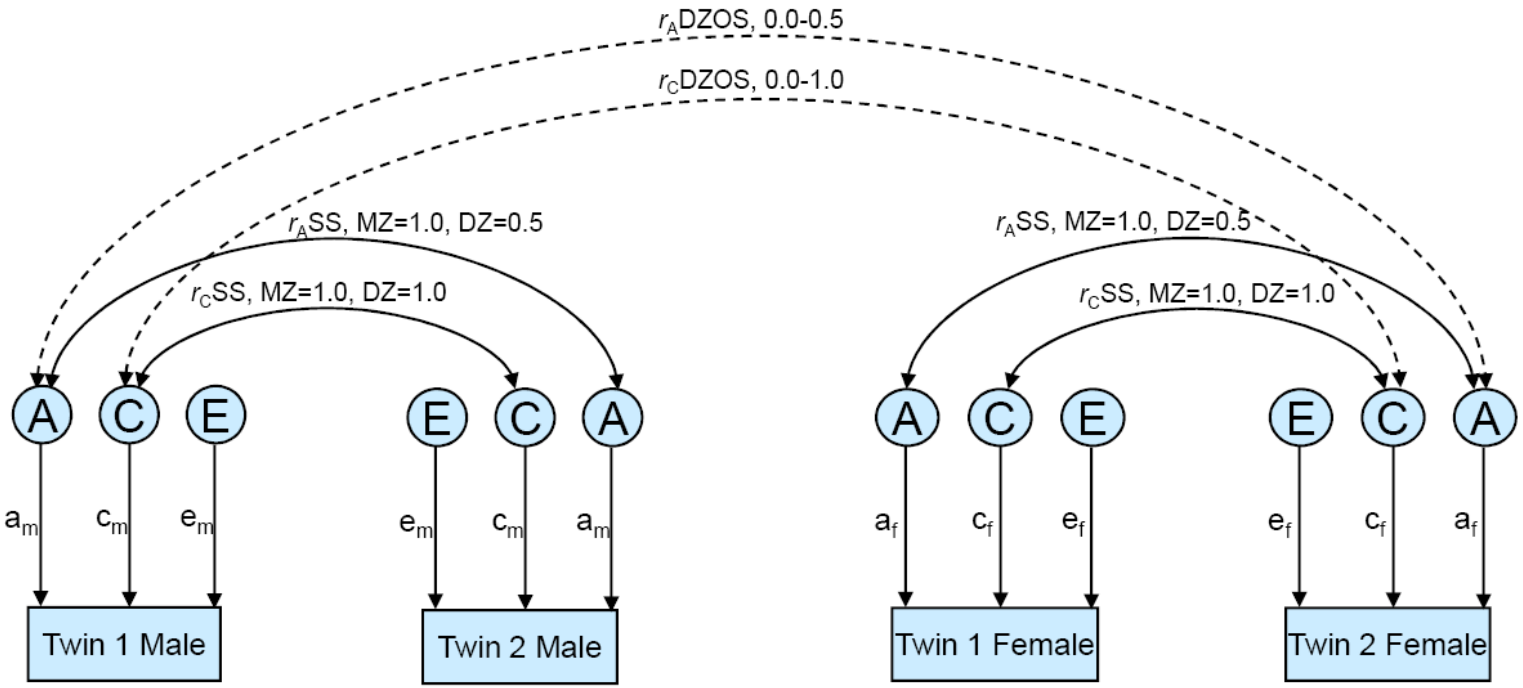
4.7.4.1. The sex-limitation model

Opposite-sex DZs make it possible to explore both 'qualitative' and 'quantitative' sex-differences in the influence of genes and the environment on the trait of interest. 'Qualitative' sex differences in A and C refer to different genes or different shared environments influencing the trait in males and females; 'quantitative' sex differences in A and C, are differences in the magnitude of genetic and shared environmental effect sizes across the sexes. To model sex-differences, the fullest model is run first which includes both types of sex differences – the 'full sex-limitation model' is shown in Figure 4.8 below. To test for qualitative *genetic* differences the model allows the genetic kinship coefficient (r_A) to vary between opposite-sex DZs from 0 to 0.5, while r_A remains fixed at 0.5 for the same-sex DZs; to test for qualitative differences in the *shared environment* effects the shared environment kinship coefficient (r_C) is allowed to vary from 0 to 1.0 for opposite-sex DZs but r_C is fixed at 1.0 for the same-sex DZs. Lower values for r_A or r_C for opposite-sex DZs indicate that different genes or different environments may influence the traits in boys and girls. The first model also allows for quantitative sex-differences in A, C and E by estimating these parameters separately for males and females. It is not possible to vary both r_A and r_C in the same model, so these are modelled separately.

Sub-models with more constraints are subsequently fit to the data; these are nested within the full sex-limitation models (the one freeing r_A for opposite-sex DZs, and the other freeing r_C for opposite-sex DZs). A 'common-effects model' is run next which allows quantitative but not qualitative sex differences; r_A is constrained to be 0.5 and r_C is constrained to be 1.0, but the effect sizes of A, C and E are allowed to differ for males and females. The 'common effects model' is then tested against the two fuller models using the LRT (and AIC, and BIC); if constraining r_A or r_C leads to a worsening of fit compared with the fullest model, then r_A and r_C should be estimated separately for males and females, indicating the presence of qualitative differences in either of these parameters.

Lastly, a 'null model' then equates A, C and E effects across males and females thereby assuming there are no differences of any kind between boys and girls. If the null model leads to a worsening of fit compared to the 'common effects' model, A, C and E should be estimated separately for males and females indicating that there are 'quantitative' sex differences in these parameters. A 'common effects model' can also be used to test for other subgroup differences – e.g. to test whether the genetic effect size on appetite differs for babies who are bottle-fed or breast-fed.

Figure 4.7. Full Sex-limitation Model



The full sex-limitation model. The path coefficients a, c, and e indicate the relative influence of the latent variables on the phenotype for males and females separately (subscript m or f, respectively) which permits the evaluation of quantitative sex differences in the effect sizes of the parameters. r_ASS is the genetic correlation for same-sex DZ and MZ twin pairs (MZ=1.0, DZ=0.5) and r_CSS is the shared environmental correlation for same-sex DZ and MZ twin pairs (MZ and DZ=1.0); r_ADZOS and r_CDZOS represent the genetic and shared environmental correlations respectively for dizygotic opposite-sex twins and may be allowed to vary to assess the likelihood of qualitative sex differences in these parameters.

4.7.5. Epistasis

Epistasis describes an interaction between alleles at different loci, as opposed to genetic dominance which refers to an intralocus interaction; both types of interaction deviate away from a purely additive model (Plomin et al., 2008). For example, an allele at one locus (e.g. A) may predispose towards obesity only if that individual also has a specific allele at another locus (e.g. B); if this is the case, the effects of different loci may not add up independently. For example, there may be two loci that each contribute 1 point to an individual's score on a trait; under a purely additive model with no epistasis having a risk allele at each loci would increase the individual's score by 2 points; on the other hand, an epistatic interaction between the two alleles may increase the individual's score by 10 points (Plomin et al., 1977a). Epistasis complicates analysis. A DZ correlation that is substantially less than half the MZ correlation may indicate epistasis, but as I explained in section 4.3 this is also suggestive of genetic dominance. There are no methods to quantify dominance versus epistasis, but a non-additivity model (ADE) can capture all non-additive genetic effects in general.

4.7.6. Gene-environment correlations

A gene-environment correlation refers to an individual's environment being related to their genotype, and Plomin and colleagues (Plomin et al., 1977a) have suggested three possible types – 'passive', evocative' and 'active'. The 'passive' type describes the scenario whereby parents bestow a particular family environment on their child that reflects the parents' own genetic proclivities as well as that of their child who has inherited both the genes and the environment (e.g. parents may cook their preferred foods for family meals, and genes are important drivers of food preferences). An 'evocative' type refers to individuals evoking particular reactions from the environment in response to their genetically-determined traits such as appearance, personality or eating behaviour (e.g. a very food responsive child may be more likely to be rewarded with food for good behaviour compared with children who are less interested in food, and their food responsiveness is ultimately driven by genes). The 'active' kind would occur if individuals were to take an

active role in choosing or seeking out environments in-line with their genetic dispositions, such as individuals choosing to live in an area with access to restaurants so that they may freely indulge in meals out, while enjoyment of food is a genetically-determined trait.

In this way, individuals who are genetically more similar (i.e. MZs versus DZs) will share more similar environments as a result of their environmental experiences being related to their genetic make-up. These differences in environmental experience do not constitute a violation of the equal environments assumption because the differences in the environmental experience are being driven by genetic differences rather than being imposed upon them by independent environmental influences (Eaves et al., 2003). These types of effects result in increased phenotypic variance in the traits (Plomin et al., 1977a). Estimating the extent of phenotypic variation accounted for by gene-environment correlations is no easy feat, especially given that there are many possible types. Standard twin models are unable to offer any real solutions (Plomin et al., 2008). Instead it is easier to examine specified gene-environment correlations, and the methods for doing this are described in detail elsewhere (Plomin et al., 2008).

4.7.7. Gene-environment interactions

A gene-environment interaction is when the effects of the environment on a phenotype depend on genetics, or conversely the effects of genetics on a phenotype depend on the environment so that the same environmental exposure may differentially affect two individuals with different genetic propensities (Kendler & Eaves, 1986; Plomin et al., 1977b). For example, a gene-environment interaction may be demonstrated if bottle-feeding infants bestows a greater risk for obesity only for infants carrying a particular allele.

Gene-environment interactions also increase phenotypic variance (Plomin et al., 2008), although again it is difficult to quantify the total amount of variation in a trait accounted for by gene-environment interactions in general (Jinks & Fulker, 1970; Plomin et al., 1977a; van der Sluis et al., 2006). Nevertheless it is possible to evaluate specific gene-environment interactions in the context of a twin study. The most straightforward method of

doing this is to assess if the genetic effect size differs significantly between groups; a model with separate A, C and E parameters for different groups (e.g. breast-fed twin pairs and bottle-fed twin pairs) can be tested against a model that combines A, C and E for the groups, and if a model with separate estimates fits the data better, there is evidence of a gene-environment interaction. An issue with this method is sample-size requirement – e.g. Purcell (2002) has estimated that about 1000 pairs of MZs and 1000 pairs of DZs would be needed to detect a heritability difference of 60% versus 40% between two groups with different environmental exposures. Gene-environment interactions are probably the rule rather than the exception in behavioural traits, as most genetically-determined behaviours depend on environmental elicitation.

4.7.8. Incorporating more complex effects

It is clear that methods are available to allow the exploration of a number of complex effects within a heritability model. However, it is not generally possible or advisable to incorporate all of these at the same time as it hinders interpretation of the findings, and the sample size needs to increase with every additional sub-group added to the analysis. Researchers in this area purport that the most fruitful approaches to analysis identify an appropriate model to use a priori using existing knowledge of the traits of interest – for example, a multivariate model of heritability is most useful when there are sound theoretical reasons for postulating shared underlying pathways, and interaction models are only of interest if there is rationale for expecting differences between sub-groups (such as mean differences observed in a trait) (Plomin et al., 2008).

4.8. Power

The statistical power for estimating heritability in a twin design is the same as any other statistical test – i.e. the probability of correctly rejecting the null hypothesis (i.e. not making a Type II error). In terms of heritability analysis this translates into correctly detecting a significant effect of a specified parameter (Schmitz et al., 1998). As with all statistical tests of significance the power to detect genetic and environment effects depends upon the effect sizes and larger samples are needed to detect smaller effects. Where heritability

studies differ from many traditional statistical analysis methods is that the number of participants required to obtain 80% power to detect significant heritability or shared environmental effects is generally very large. Nevertheless, far fewer participants are needed if additive genetic effects or shared environmental effects are very high. The number of participants needed to detect a given additive genetic effect always depends on the size of the shared environmental effect as well, and vice versa. Posthuma and Boomsma (2000) have published data on the total number of individual twins that are needed for 80% power to find significant additive genetic and shared environmental effects of differing proportions, at an alpha level of 0.05, and assuming an MZ/DZ ratio of 1:1. Table 4.3 shows the numbers of individual twins needed to detect varying additive genetic effects (from 10% to 90%) in the context of shared environmental effects of 0%, 10% and 20%. Table 4.4 shows the numbers needed to detect varying shared environmental effects in the context of additive genetic effects. An important empirical fact that they highlight is that shared environmental influences tend to be small, while additive genetic influences tend to be fairly high, so they also provided information on numbers needed to detect shared environment effects of 10% and 20% in the presence of additive genetic effects up to 80%.

However, researchers in this area have used data simulation to show that there ways of increasing power other than recruiting numerous twins. Firstly, increasing the ratio of DZs to MZs increases power (e.g. from 1:1 to 1:2, or even better to 1:4) (Neale & Maes, 2001), and this is facilitated by there being more DZs than MZs in most populations. Another option is to include other siblings in the family in the analysis (Posthuma & Boomsma, 2000). A much more straight forward method is to include other correlated variables in the analysis – when variables are correlated with one another multivariate models have more power than univariate models because they use phenotypic covariances to assist in estimating A, C and E (Schmitz et al., 1998). Schmitz, Cherny and Fulker (1998) have also provided power calculations for differing levels of genetic and environmental effects with given sample sizes (200, 300 and 400 individual twins with an MZ:DZ ratio of 1:1) and an alpha level of 0.05. They estimated that in the three variable case a sample of only 300 subjects is sufficiently powered (82%) to detect heritability of only 30%, while a four variable model only requires 200 participants to detect this effect with nearly 80% power (0.75) (in each case assuming shared environmental effects of 20%, shared environmental

correlations to be 0.80, genetic correlations to be 0.50 and no unique environmental correlations).

Table 4.3. Total number of individual twins required to detect additive genetic effects at varying levels of shared environmental effects with an MZ:DZ ratio of 1:1 (data from Posthuma & Boomsma, 2000)

Additive genetic effects	Shared environmental effects		
	0%	10%	20%
10%	24896	23084	20110
20%	5908	5230	4332
30%	2406	2026	1588
40%	1192	950	700
50%	644	482	328
60%	360	248	150
70%	198	124	60
80%	104	52	-[a]
90%	48	-[a]	-

[a] No estimates are reported for 80% additive genetic effects and 20% shared environment effects or 90% additive genetic effects and 10% shared environmental effects because error must be included in the model (subsumed under unique environment effects).

Table 4.4. Total number of individual twins required to detect shared environmental effects at varying levels of additive genetic effects with an MZ:DZ ratio of 1:1 (data from Posthuma & Boomsma, 2000)

Shared environmental effects	Additive genetic effects								
	0%	10%	20%	30%	40%	50%	60%	70%	80%
10%	15504	14860	13934	12806	11558	10280	9042	7912	6934
20%	3646	3398	3104	2786	2466	2158	1876	1628	-[a]
30%	1468	1334	1190	-	-	-	-	-	-
40%	722	640	560	-	-	-	-	-	-
50%	390	340	290	-	-	-	-	-	-
60%	222	188	156	-	-	-	-	-	-
70%	126	104	84	-	-	-	-	-	-
80%	70	54	-[a]	-	-	-	-	-	-
90%	36	-[a]	-[a]	-	-	-	-	-	-

[a] No estimates are reported for 80% additive genetic effects and 20% shared environment effects or 90% additive genetic effects and 10% shared environmental effects because error must be included in the model (subsumed under unique environment effects).

4.9. Generalisability from twins to singletons

Twins sometimes differ from singletons on certain characteristics, particularly in early life. Twins are generally born 3-4 weeks earlier than singletons (Phillips, 1993), have a lower birth weight (MacGillivray et al., 1988), and experience more rapid growth during early life than singletons in order to 'catch-up' (Naeye et al., 1966). However, being a twin or a singleton does not appear to impact on individual differences across other areas such as personality (Johnson et al., 2002), motor development (Brouwer et al., 2006) and psychopathology (Christensen et al., 1995). While the literature in this area does indicate that generalisability of findings from twins to singletons cannot be taken for granted, it is also clear that for many traits being a twin is no different from being a singleton. Where there are known differences between twins and singletons, sensitivity analyses can be run to test if these characteristics are influencing heritability estimates unduly (e.g. run a heritability analysis with all twins and then excluding twins who were extremely premature).

CHAPTER 5. SAMPLING AND METHODOLOGY: GEMINI – HEALTH AND DEVELOPMENT IN TWINS[22]

5.1. Overview of Gemini[23]

Gemini is a prospective study using a birth cohort of twins set up in 2007 by Professor Jane Wardle within the Department of Epidemiology and Public Health at University College London to assess the genetic and environmental influences on growth during the first 5 years of life, with a focus on infant appetite, activity preference and the family food and activity environments (van Jaarsveld et al., 2010). The research aims of the study are threefold: (i) to advance understanding of the genetic and environmental influences on weight gain; (ii) to identify modifiable determinants of excessive weight gain in early childhood, and; (iii) to create a rich resource of data on early childhood exposures that can be used to assess the determinants of long-term health. In particular, the study is focusing on the behavioural mechanisms behind weight gain (such as an avid appetite), and will characterise the extent to which 'obesogenic' behavioural traits are associated with different rearing environments (such as the early milk-feeding regimen and the later food environment).

5.2. Methods

5.2.1. Sample and recruitment

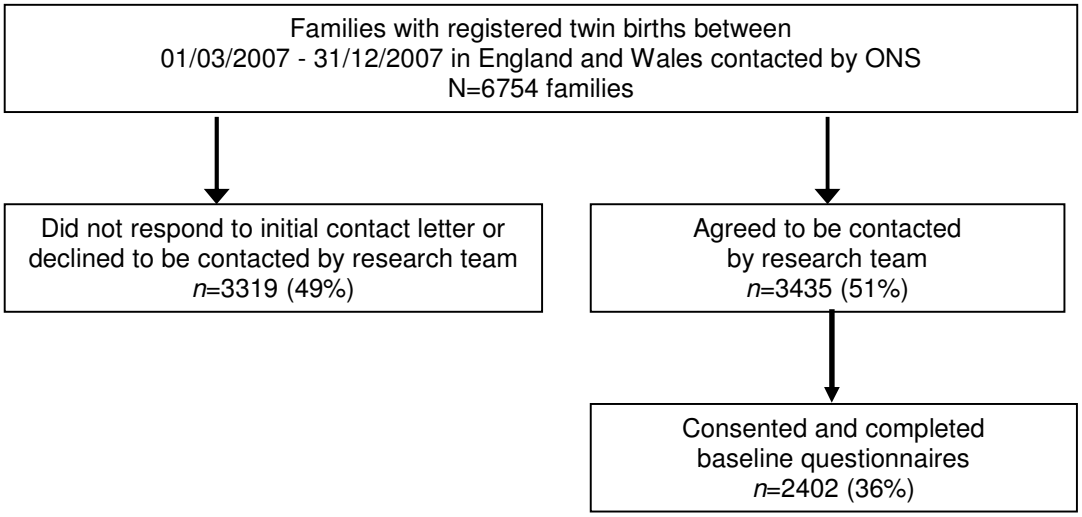
All families with twins born in England and Wales between March and December 2007 were eligible to take part in Gemini, if the mother and both twins were alive in January 2008. At this point in time, the government agency responsible for birth registration (the Office for National Statistics [ONS]) wrote to all eligible families with twins (N=6754), to ask

[22] Much of the information in this chapter has now been published in the following paper: van Jaarsveld CH, Johnson L, Llewellyn C and Wardle J. (2010). Gemini: a UK twin birth cohort with a focus on early childhood weight trajectories, appetite and the family environment. *Twin Research and Human Genetics*, 13, 72-8.

[23] Materials for the Gemini study are shown in Appendix 3.

for their consent to pass their contact details on to our research department[24]. Data was cross-linked with data from the National Health Service Central Registry (NHSCR) to verify that the mother and both twins were alive. 51% of the families (*n*=3435) confirmed in writing that they were willing to be contacted by our research team. Between February and July 2008, all of these families were sent the baseline questionnaire, together with an information leaflet giving details of the study, and a consent form, to be sent back with the questionnaire[25]. Two reminder letters were sent following the baseline questionnaire. 2402 families agreed to take part and completed and returned the baseline questionnaire; these included 36% of families initially contacted by ONS or 70% of the families that consented to being contacted by our research team (Figure 5.1). An initial response rate of 36% to ONS was considered reasonable taking into account the fact that families had infant twins less than 9 months old at the time they were contacted. A final response rate of 70% was as expected, considering that the families were asked to complete a lengthy questionnaire about a number of different topics related to their twins and the wider family. Participating families live across the whole of England and Wales. Ethical approval for Gemini was granted by University College London Committee for the Ethics of non-NHS Human Research.

Figure 5.1. Flow diagram of recruitment of Gemini families



[24] The initial invitation letter is shown in Appendix 3.1.
[25] The letter that was sent to the families with the baseline questionnaire is shown in Appendix 3.2, the consent form in Appendix 3.3, the information leaflet in Appendix 3.4 and the baseline questionnaire in Appendix 3.5.

5.2.2. Data collection

All data for Gemini was collected via the parents. The main method of data collection was parent-report questionnaire, and the questionnaire was available both in hard copy and online via the internet (9.7% of families completed the baseline questionnaire online). This thesis uses data collected from the baseline questionnaire which was sent to the families between February and July 2008 when the twins were about 8 months old. All measures that were designed for Gemini were intensively piloted in parents of young children (both singletons and twins). All other measures were based on validated questionnaires.

5.2.2.1. Baseline questionnaire measures

The parent who completed the baseline questionnaire was asked to state her/his relationship to the twins and completed information about her/himself as well as her or his cohabiting partner, if applicable. The first half of the questionnaire included questions about the twins' age and zygosity, the mother's pregnancy and birth, parental anthropometrics, health behaviours, ethnicity and sociodemographics, as well as information on the weight and health of the wider family. The other half focused on the twins and included anthropometrics from birth, appetite and feeding behaviour, food preferences, activity behaviour and parental feeding style. The measures included in this thesis are described in more detail below.

5.2.2.1.1. Twin zygosity

5.2.2.1.1.1. Zygosity questionnaire[26]

Parents were asked whether their twins were opposite-sex or the same sex. Opposite-sex twins were classified as DZ. Parents of same-sex twins were asked to complete a set of 20 questions originally developed to establish the zygosity of 18-month old twins in the Twins Early Development Study (Price et al., 2000). The questionnaire has performed well when validated against polymorphic DNA markers demonstrating 95% accuracy, and has been

[26] Twin zygosity coding using the questionnaire was performed by Ellen van Jaarsveld.

shown to be reliable over time with 96% of twins being assigned the same zygosity at 18-months and 3 years of age (Price et al., 2000).

A number of questions relate to physical resemblance including general likeness (e.g. 'Would you say that your twins are: (i) as physically alike as 'two peas in a pod'; (ii) are as physically alike as brothers and sisters are, or; (iii) do not look very much alike at all?'), and specific features known to be highly influenced by genes such as hair colour and texture, eye colour, ear lobe shape, and timing of teeth coming through (e.g. 'Are there differences in the shape of your twins' ear lobes?'). Other items ask about blood type and ease with which parents, friends and other family members can distinguish the twins (e.g. 'When looking at a new photograph of your twins, can you tell them apart without looking at their clothes or using any other clues?'). One question asks about healthcare professional opinion on their zygosity, and another asks about the parents' own opinion on their zygosity (e.g. 'Do you think your twins are identical or non-identical?').

Zygosity was determined through two possible methods. Certain individual items in the questionnaire held greater weight in assigning zygosity status, such that the response to one of these items alone was used to classify the twins. Twin pairs described as 'two peas in a pod' were classified as MZ as this question alone has been shown to correctly classify a high proportion of MZ twin pairs (Cederlof et al., 1961). Twin pairs described as 'not looking much alike at all' or as having clear differences in eye colour, hair colour or hair texture were classified as DZ, except where they were described elsewhere as being like 'two peas in a pod', in which case they were not classified using this system, but were instead classified using the scoring system described below. Twin pairs whose blood types were discordant were classified as DZ.

For all other cases, twin pairs were classified using a scoring system, based upon the responses to the items. A total score was calculated for each twin pair by adding up the scores obtained for each question and dividing the total by the maximum possible score based upon the number of questions answered, to create a value between 0 and 1. The lower the score, the greater the intra-pair similarity with 0 representing maximal similarity; likewise, higher scores denote more dissimilarity with 1 representing maximal dissimilarity. All scores $\leq$ 0.64 were classified as MZ, all scores $\geq$ 0.70 were classified as DZ. Scores > 0.64 and < 0.70 were coded as having 'unknown' zygosity in-line with the instructions

provided in the paper describing the development and validation of the questionnaire (Price et al., 2000). In addition, twin pairs who had missing data for 50% of the items or more were classified as having 'unknown' zygosity.

5.2.2.1.1.2. DNA validation of the zygosity questionnaire

All of the Gemini families were invited to provide DNA for each of their twin children in order to measure molecular genetic variants of interest for the study at a later stage. DNA was collected by the parents using cheek swabs that were sent and returned by post. In order to check the validity of the zygosity questionnaire a random sample of 10% of the Gemini families who returned the DNA (*n*=81 pairs; 43 MZ pairs, 38 DZ pairs) were zygosity-tested using the twins' DNA, the processing of which was carried out at a laboratory at the Institute of Psychiatry.

5.2.2.1.1.3. Parental misclassification of zygosity

In the zygosity questionnaire parents were asked if they thought their twins were identical or non-identical. This item was used to sub-categorise zygosity further into twin pairs who were classified the same by both the zygosity questionnaire and the parents and those whose questionnaire classification and parental classification differed, giving rise to 4 possible groups: (1) pairs classified as MZ by both the questionnaire and the parents [MZQ-MZP]; (2) pairs classified as MZ by the questionnaire and as DZ by the parents [MZQ-DZP]; (3) pairs classified as DZ by both the questionnaire and the parents [DZQ-DZP]; (4) pairs classified as DZ by the questionnaire and as MZ by the parents [DZQ-MZP].

5.2.2.1.2. Parent and infant anthropometrics[27]

5.2.2.1.2.1. Infants

Weight data at birth and in the first months after birth was collected in the baseline questionnaire. Parents reported their children's weights from birth onwards using measurements made by health professionals that were recorded in the child's personal health record ('red book'). Parents were asked to photocopy the relevant pages of their child's red book or copy all available measurements for each twin into the questionnaire. When health professional weight measurements were unavailable, parents were asked to record weight measurements made by themselves (3.6% of data). The option was given to provide anthropometric data in imperial or metric units. All imperial data were later converted to metric, and in cases where information was provided in both measurement units the metric units were used. Birth weights less than 0.5 kgs and greater than 5.0 kgs were considered misreports and were coded as missing.

Weight at 3 months was derived for each twin by selecting the measurement occasion that occurred closest to 3 months within the range of -1 to +1 month of age. Exact age at the measurement occasion closest to 3 months was calculated. Growth curves were plotted for each infant using all of the weight data from birth onwards and any weights that were gross outliers were considered misreports and coded as missing. Weight standard deviations scores (SDS) at birth and 3 months and were calculated adjusting for exact age (at 3 months), sex and gestational age based on British 1990 growth reference data using the LMSgrowth macro for Microsoft Excel (Cole, 2009; Freeman et al., 1995). A weight SDS of 0 indicates average weight, a SDS > 0 indicates a higher weight and a SDS < 0 indicates a lower weight compared to the 1990 growth reference (Freeman et al., 1995).

[27] Weight SD scores were calculated by Ellen van Jaarsveld.

5.2.2.1.2.2. Parents

Heights and weights of both parents were self-reported. The body mass index (BMI) of each parent at the time the questionnaire was completed was calculated using the following equation: weight (kg) / height $(m)^2$.

5.2.2.1.3. Sociodemographic information

5.2.2.1.3.1. Age

We obtained two different indices of infant age: infant age at the time the questionnaire was completed was calculated in months and days (indicated as a whole number and a decimal) using the twins' date of birth and the date upon which the questionnaire was completed. We asked the parents to report the number of weeks the mother had been pregnant at the time of delivery and this was used as an estimate of gestational age. The age of each parent at the birth of the twins was calculated in years and days (indicated as a whole number and a decimal) using the twin's and parents' dates of birth.

5.2.2.1.3.2. Biological parentage and marital status

The parent completing the questionnaire was asked to give information about their relationship to the twins (e.g. 'natural mother', 'natural father' or 'legal guardian of the twins'), and to state their marital status using one of the following categories: 'married or cohabiting', 'divorced', 'widowed', 'separated', 'single'. The categories were later collapsed into 'married or cohabiting', 'divorced or separated' or 'single', based upon numbers and conceptual distinction.

5.2.2.1.3.3. Socioeconomic status

Family social class was indexed using a number of different indices. Parents reported on their highest educational qualification from seven options: 'No qualifications', 'CSE, GCSE

or ‘O’ level’, ‘Vocational qualification (GNVQ, BTEC)’, ‘’A’ or ‘AS’ level’, ‘Higher National Certificate (HNC) or Diploma (HND)’, ‘Undergraduate degree’, ‘Postgraduate qualification (Masters, PhD)’. Education level was later collapsed into three categories: ‘low’ included ‘No qualifications’ and ‘CSE, GCSE or ‘O’ level’; ‘middle’ included ‘Vocational qualification (GNVQ, BTEC)’ and ‘’A’ or ‘AS’ level’; ‘high’ included ‘Higher National Certificate (HNC) or Diploma (HND)’, ‘Undergraduate degree’ and ‘Postgraduate qualification (Masters, PhD)’. Parents were also asked to report on their employment status by stating whether they were on maternity leave (mothers only), in full-time employment, in part-time employment, not in employment, or staying at home to look after the children.

Parents were also asked to describe their occupation and that of their partner and this was used to calculate the National Statistics Socioeconomic Class (NS-SEC) index. It was derived using the simplified method described by the ONS (Office for National Statistics, 2005) – the Computer-Assisted Structured Coding Tool (Jones & Elias, 2005) was used to assign job descriptions to their corresponding four digit Standard Occupational Classification 2000 code (Office for National Statistics, 2000a; Office for National Statistics, 2000b). These codes were linked to a reversed eight category NS-SEC classification, so that higher scores represent higher SES. To determine household SES, a household reference person was defined by selecting the person with the highest SES, which was the partner, the mother and was equal in 41%, 29% and 18% of families, respectively. In the remaining 12%, where data were missing or the mother did not have a partner, the person that did have SES data was assigned as household reference person. In order to have adequate group sizes for analysis, NS-SEC scores were grouped into higher (higher and lower managerial and professional occupations), intermediate (intermediate occupations, small employers and own account workers – self-employed with no employees) and lower SES (lower supervisory and technical occupations, (semi-)routine occupations, never worked and long-term unemployed) (Office for National Statistics, 2005) [28].

In addition, parents were asked to report the total gross income of the whole household per year, ranging from “Up to £15,000 per year” to “more than £90,000 per year”, with 12 response options which were later collapsed into 5 categories, based on numbers. Home

[28] NS-SEC coding was carried out by an MSc student working on the Gemini study.

ownership (“own without mortgage”, “own with mortgage”, “rent privately”, or “rent from local authority”), number of bedrooms per household, and number of cars per household were also ascertained.

5.2.2.1.3.4. Ethnicity

Parents were asked to select their ethnicity and that of their partner from 16 possible categories taken from ONS’s National Statistics interim standard classifications for presenting ethnic and national groups data (categories include ‘White British’, ‘White Irish’, ‘Other White background’, ‘Caribbean’, ‘African’, ‘Other Black background’, ‘Indian’, ‘Pakistani’, ‘Bangladeshi’, ‘Other Asian background’, ‘White and Black Caribbean’, ‘White and Black African’, ‘White and Asian’, ‘Other Mixed background’, ‘Chinese’, ‘Any other’. In all cases of ‘other…’ parents were asked to specify their ethnicity). Categories were later collapsed into ‘White-British’ and ‘non White-British’ (and ‘unknown’ in the cases of missing data) as numbers across the non White-British categories were too small to allow for meaningful sub-group analyses among other ethnic groups.

Parental ethnicity was used to classify twin ethnicity: if both parents selected the same category the twins’ ethnicity was classified using that category; if parents selected different categories the twins were classified as of ‘mixed ethnicity’; if only one parent’s ethnicity information was missing, twin ethnicity was classified using the other parent’s ethnicity group. In the final analysis twin ethnicity was also collapsed into ‘White-British’ and ‘non White-British’ due to the small numbers of twins in non White-British categories; there was ethnicity information for all twins using at least one parent’s information.

5.2.2.1.3.5. Parental health behaviours

Information about three health behaviours was also obtained for both parents. Respondents were asked if they smoked cigarettes currently (‘Do you smoke cigarettes at all nowadays?’, ’yes’ or ‘no’), and mothers were asked if they smoked at all during their pregnancy (‘Did you smoke any cigarettes whilst pregnant?’, ‘yes’ or ‘no’?). Parents also reported how many servings of fruits and vegetables they ate in the last week ranging from ‘less than 1 per week’ to ‘4 or more per day’, with 8 response options in total based upon

those used in the European Prospective Investigation of Cancer study (Sargeant et al., 2001). In order to estimate total consumption of fruits and vegetables, each participant received a score for their fruit and vegetable consumption separately by recoding the categories using the following scoring system: 1=0.1, 2=0.2, 3=0.4, 4=0.8, 5=1, 6=2, 7=3, 8=4. The score represented the portion of fruit or vegetables consumed in one day; adding the two scores together then gave an estimation of the total number of portions of fruit and vegetables consumed by each parent during one day[29].

5.2.2.1.4. Infant appetite and feeding method

5.2.2.1.4.1. Infant appetite

Parents completed the Baby Eating Behaviour Questionnaire (BEBQ) for each twin, an 18-item measure of appetite developed for Gemini which focuses on the earliest period of life during the milk-feeding phase. Parents were instructed to think back to the first 3 months of their infant's life during the period when they were fed milk only, when responding. The development of this measure is the focus of the first study and is described in detail in Chapter 6.

5.2.2.1.4.2. Feeding method

Infant feeding methods used during the first 3 months were assessed by asking mothers to report the proportion of breast-feeding versus bottle-feeding, using the question: 'Which feeding methods did you use in the first three months', with response options: 'entirely breastfeeding'; 'mostly breastfeeding with some bottle-feeding'; 'equally breastfeeding and bottle-feeding'; 'mostly bottle-feeding and some breastfeeding'; 'almost entirely bottle-feeding (only tried breastfeeding a few times)'; 'entirely bottle-feeding (never tried breastfeeding)'; and 'other'.

[29] Fruit and vegetable consumption scores were calculated by Ellen van Jaarsveld.

5.2.2.1.4.3. Infant feeding problems

Parents were asked two questions about feeding problems ('yes' or 'no'): 'Straight after birth, did either of your twins experience any complications which made it difficult to start feeding', and 'Were there any other times when feeding your twins was difficult, e.g. due to illness of the twins, health problems of the parent, changes on jobs or moving house.' Infants were divided into those with no reported feeding problems at any time (parent answered 'no' to each question) and those with any reported feeding problem (parents answered 'yes' to at least one of the questions).

5.2.3. Non-response analyses and Gemini representativenesss

Non-response analyses were conducted on three variables which were provided by ONS (the month of the twins' birth, the mother's age at the twins' birth, and the region of residence) for all families they contacted in 2007. Pearson's chi-square tests were used to assess differences between the target population and the Gemini cohort. The representativeness of the Gemini cohort was assessed by comparing the sociodemographic characteristics of the sample measured in the baseline questionnaire with that of the wider population using national statistics published by ONS[30].

5.2.4. Zygosity questionnaire validation and tests for zygosity differences

Percentages of twin pairs classified as the same or different by the questionnaire and the DNA test were calculated to assess the validity of the zygosity questionnaire. Zygosity differences across all sociodemographic measures were tested. For continuous measures Independent Groups t-tests were used, and for categorical measures Pearson's chi-square test was used; in each case Cohen's *r* was calculated for significant differences as a measure of the effect size[31].

[30] This analysis was performed by Ellen van Jaarsveld.
[31] Small effect size, r=0.1–0.23; medium, r=0.24–0.36; large, r=0.37 or larger (Cohen, 1988; Cohen, 1992).

5.2.5. Tests for twin design assumptions

As highlighted in Chapter 4, a number of assumptions are inherent in quantitative genetic models. Some of these can be tested directly in the genetic structural equation models (e.g. heterogeneity, gene-environment interactions, sibling interaction effects, parental rating biases, and non-additivity) but others cannot, including random (rather than assortative) mating among the parents and testing of equal environments for MZ and DZ twins. Assortative mating for adiposity was assessed by testing the association between parental weight and BMI, using a Pearson's Product Moment correlation coefficient. Testing the equality of environments for MZs and DZs is a more challenging task. However, from the available measures one possibility was to test for differences by zygosity in concordances for feeding method[32] and feeding problems, as concordance differences could indicate greater mismatch in the environments shared by MZ or DZ pairs. Pearson's chi-square test was used to test whether the proportion of MZ and DZ twins who were both mainly breast-fed ('entirely breastfeeding' and 'mostly breastfeeding with some bottle-feeding'), mixed-fed ('equally breastfeeding and bottle-feeding'), or mainly bottle-fed ('mostly bottle-feeding and some breast-feeding', 'almost entirely bottle-feeding', and 'entirely bottle-feeding') was different, as well as concordances for feeding problems.

5.2.6. Power

Heritability power calculations based on the sample size of 729 MZ pairs and 1605 DZ pairs were conduced in Mx (version 32; Virginia Commonwealth University, Richmond, VA) for an alpha level of 0.01 (taking account of the large sample size) for univariate analyses. Power calculations for multiple regression analyses were calculated using G-Power (version 3.0.10; Softpedia) based on varying numbers of infants, for models including 10 predictor variables, at an alpha level of 0.01.

[32] Although finding higher differences in feeding method could also indicate evocative gene-environment correlations.

5.3. Results

5.3.2 Representativeness of the Gemini sample

5.3.2.1 Comparisons between responders and non-responders

Table 5.1. shows the ONS information for the target population and those who responded (the Gemini families). Response rates ranged from 32% to 42% by month of twins' birth (χ^2=21.187 (9*df*), *p*=0.0118) with somewhat higher response rates among more recent births (November) and lower response rates for births earlier in the year (March and April). Response rates ranged from 23% to 45% by mother's age at the twins' birth (χ^2=151.447 (5*df*), *p*<0.001), with higher response rate in 30-34 year olds and lower response rates in younger (20-24 years) and older (over 40 years) age groups. Lastly, response rates ranged from 19% to 45% by region of residence (χ^2=241.261 (9*df*), *p*<0.001), and were higher in the South East of England, the East of England, the Midlands, and the South West of England, and lowest in the London area. Figure 5.2 shows the distribution of the Gemini families across England and Wales.

Table 5.1. Non-response analyses comparing families participating in Gemini with the target population

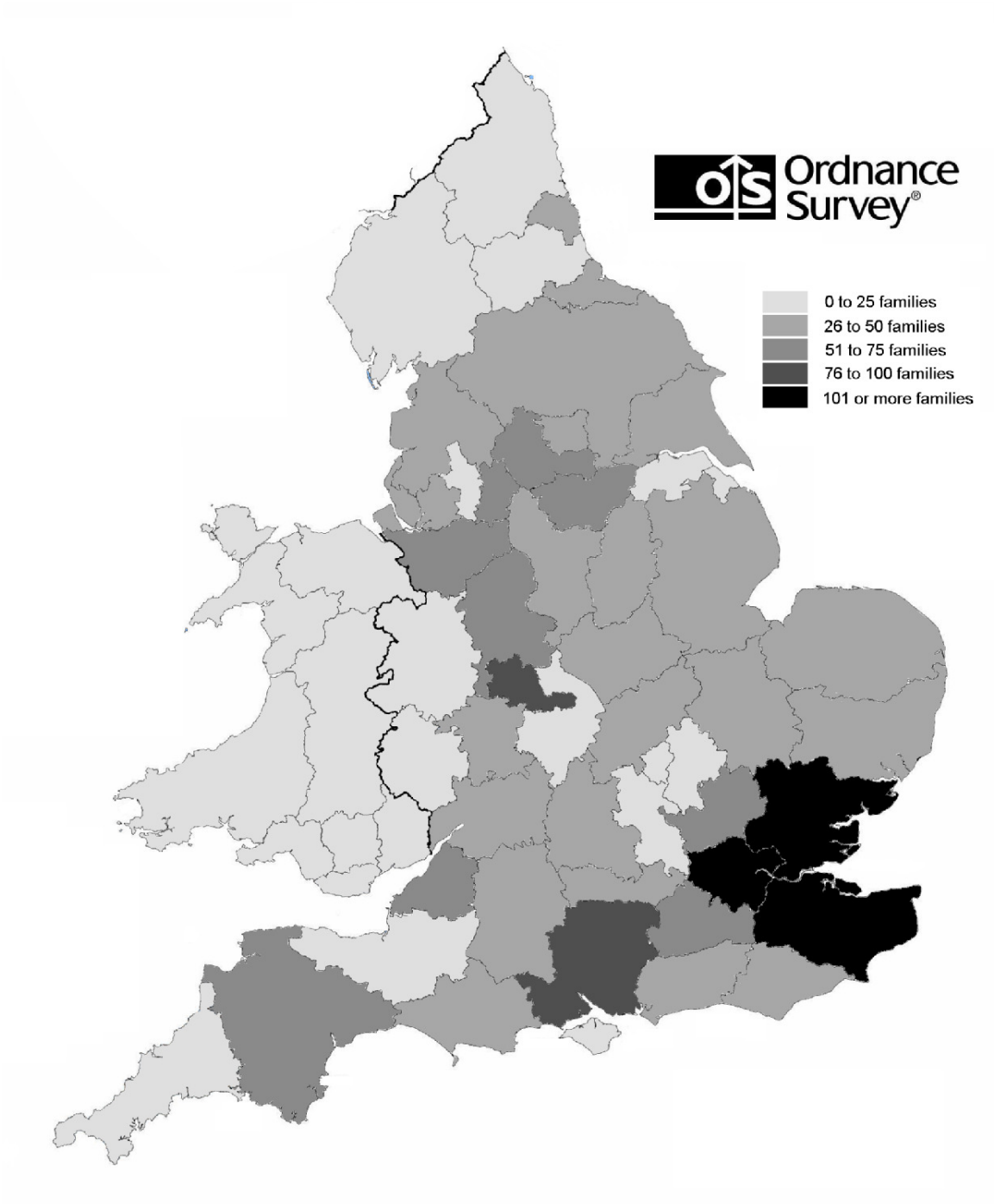
	Target population *n*=6754 [a]	Gemini families *n*=2402	Response %
Month of twins' birth (all in 2007)			
March	**766**	**245**	**0.32** [b]
April	**720**	**238**	**0.33** [b]
May	**776**	**277**	**0.36**
June	**773**	**282**	**0.36**
July	**861**	**296**	**0.34**
August	**677**	**244**	**0.36**
September	**718**	**252**	**0.35**
October	**729**	**261**	**0.36**
November	**616**	**261**	**0.42** [c]
December	**118**	**46**	**0.39**
Mother's age at twins' birth			
Under 20 years	**82**	**25**	**0.30**
20-24 years	**594**	**160**	**0.27** [b]
25-29 years	**1345**	**446**	**0.33**
30-34 years	**1993**	**900**	**0.45** [c]
35-39 years	**1995**	**714**	**0.36**
Over 40 years	**667**	**151**	**0.23** [b]
Not known	**78**	**6**	**-**
Region of residence			
London	**1209**	**231**	**0.19** [b]
South East	**1057**	**468**	**0.44** [c]
North West	**824**	**275**	**0.33**
West midlands	**712**	**228**	**0.32**
East of England	**699**	**317**	**0.45** [c]
Yorkshire and the Humber	**634**	**222**	**0.35**
East Midlands	**468**	**194**	**0.41** [c]
South West	**567**	**255**	**0.45** [c]
Wales	**320**	**117**	**0.37**
North East	**262**	**94**	**0.36**
Not Known	**2**	**1**	**-**
Total	**6754**	**2402**	**0.36**

[a] The target population consisted of 6754 families with registered twin births in England or Wales between March and December 2007 which were contacted by Office of National Statistics.

[b] Groups with much lower response rates compared to overall mean of 36%.

[c] Groups with much higher response rates compared to overall mean of 36%.

Figure 5.2. Map of England and Wales showing the distribution of families participating in Gemini



5.3.2.2 Representativeness of the Gemini cohort

Table 5.2 shows the sociodemographic characteristics of the twins compared to national statistics. The comparisons did not highlight major concerns about the ability of the Gemini cohort to represent the target population, although slight differences were observed. In summary, the Gemini study includes twins that are comparable in sex (Office for National Statistics, 2006a; Office for National Statistics, 2001b), zygosity, gestational age (Office for National Statistics, 2006a), and birth weight (Office for National Statistics, 2006a) to national averages of twins. In addition, similar rates of exclusive breast- and bottle-feeding rates were observed as in the population at large (Infant Feeding Survey, 2007). A slightly lower proportion of twins were of White-British origin than in the wider population (Office for National Statistics, 2001a).

Table 5.3 shows comparisons between the sociodemographics of the Gemini parents and national statistics. These comparisons indicated that Gemini mothers tended to be somewhat older at the twins' birth (Office for National Statistics, 2006b), and both parents were slightly healthier than the national population with lower BMIs, less current smokers, less mothers smoked during their pregnancy, and mothers had a slightly higher consumption of at least 5 portions of fruit or vegetables per day (Health Survey for England, 2008). In addition, Gemini has an over-representation of White-British married couples (Office for National Statistics, 2006b; Office for National Statistics, 2007). Indicators of socioeconomic status suggested that the Gemini parents score more highly than the national average. Fewer families rented their accommodation from a local authority, and a larger number of families owned their property with a mortgage, although compared with the population as a whole few owned their properties (Office for National Statistics, 2007). Fewer Gemini families did not own a car and a larger number owned at least 2 cars (Office for National Statistics, 2007). Educational attainment was higher than average (Department for Innovation, 2008), and a greater proportion of the families than the population at large were categorised to be of 'higher' socioeconomic status according to NSSEC, and fewer were categorised as 'lower' (Office for National Statistics, 2003).

Table 5.2. Baseline characteristics of twins participating in Gemini compared to national statistics for twins

Characteristic	Total Gemini Sample (*n*=4804 twins)			National statistics
	n (%[a]) or mean (sd)			% or mean
Weight at birth (kgs)	2.46	(0.54)		2.50[b]
Gestational age at birth (weeks)	36.2	(2.49)		37[b]
Twin age (months)	8.2	(2.16)		
Zygosity of twin pairs[g]				
DZOS	816	(34.0)	*(35.0)*	-[c]
DZF	389	(16.2)	*(16.7)*	
DZM	400	(16.6)	*(17.1)*	
MZF	384	(16.0)	*(16.5)*	
MZM	345	(14.4)	*(14.8)*	
Not known	68	(2.8)		
Sex of twin pairs				
Males	785	(32.7)		32.1%[b]
Female	801	(33.3)		32.8%
Male-female	816	(34.0)		35.1%
Sex of infants				
Males	2386	(49.7)		51.2%[d]
Females	2418	(50.3)		48.8%
Ethnicity of twin pairs				
White-British	1970	(82.0)		87.5%[e]
Non White-British	432	(18.0)		12.5%
Premature pairs (< 37 weeks)				
Premature	1045	(43.5)	*(43.7)*	40.0%[b]
Not premature	1347	(56.1)	*(56.3)*	60.0%
Not known	10	(0.4)		
Feeding method of infants				
Entirely breast-fed	676	(14.1)	*(14.9)*	14%[f]
Mostly breast-fed	895	(18.6)	*(19.6)*	-
Equally breast- and bottle-fed	446	(9.3)	*(9.7)*	-
Mostly bottle-fed	783	(16.3)	*(17.1)*	-
Almost entirely bottle-fed	686	(14.3)	*(15.0)*	-
Entirely bottle-fed	1090	(22.7)	*(23.7)*	23%
Not known/ other	228	(4.8)		
Feeding problems of infants				
No	2971	(61.8)	*(61.9)*	-[c]
Yes	1831	(38.1)	*(38.1)*	
Not know	2	(0.0)		

[a] Italicized percentages are the valid percentages which may be compared to national statistics; non-italicized percentages include missing data. Percentages may not add up to 100 due to rounding.

[b] Office for National Statistics (2006). Birth Statistics Series FM1 no.35. Review of the Registrar General on births and patterns of family building in England and Wales. Newport. (Numbers are for twin births in 2006).

[c] ONS has not published national statistics for these variables.

[d] Office for National Statistics (2001). UK census data: Population pyramids – age 0-4 years.

[e] Office for National Statistics (2001). UK census data: A guide to comparing 1991 and 2001 Census ethnic group data.

[f] Infant Feeding Survey 2005 (2007).Incidence, prevalence and duration of breastfeeding. The Information Centre for Health and Social Care. This survey reported that in 2005, 77% of mothers in England and Wales breast-fed initially (even if this was on one occasion only), so 23% of infants were never breast-fed (corresponding to our 'entirely bottle-fed'), during the first 10 weeks of life. They also reported that 14% of infants were exclusively breast-fed for the first three months of life.

[g] DZOS, dizygotic opposite-sex; DZF, dizygotic female; DZM, dizygotic male; MZF, monozygotic female; MZM, monozygotic male.

Table 5.3. Baseline characteristics of parents participating in Gemini compared to National statistics

	Total Gemini Sample (*n*=2402 families; *n*=4804 twins)			National statistics
	n (%[d]) or mean (sd)			% or mean
Mother's Ethnicity				
White-British	2089	(87.0)	*(87.0)*	78.1%[a]
Non White-British	311	(12.9)	*(13.0)*	21.9%
Not known	2	(0.1)		
Father's Ethnicity				
White-British	1988	(87.8)	*(87.8)*	72.6%[a]
Non White-British	275	(11.4)	*(12.2)*	27.4%
Not known	139	(5.8)		
Marital status				
Married or cohabiting	2276	(94.8)	*(94.8)*	60.0%[c]
Divorced or separated	31	(1.3)	*(1.3)*	10.0%
Single	93	(3.9)	*(3.9)*	20.0%
Not known	2	(0.1)		
Maternal Education				
No qualifications	129	(5.4)		11.9%[f]
CSE, GCSE, O-level, or Vocational Qualification	763	(31.8)		40.0%
A or AS-level	258	(10.7)		16.9%
HNC/HND, or Undergraduate Degree	865	(36.0)		24.3%
Postgraduate	387	(16.1)		6.9%
Paternal Education				
No qualifications	238	(9.9)	*(10.5)*	11.1%[f]
CSE, GCSE, O-level, or Vocational Qualification	842	(35.0)	*(37.0)*	36.3%
A or AS-level	166	(6.9)	*(7.3)*	22.3%
HNC/HND, or Undergraduate Degree	723	(30.1)	*(31.8)*	23.0%
Postgraduate Degree	307	(12.8)	*(13.5)*	7.3%
Not known	126	(5.2)		
NS-SEC				
Lower	472	(19.7)	*(19.7)*	33%[g]
Intermediate	407	(16.9)	*(17.0)*	18%
Higher	1515	(63.1)	*(63.3)*	49%
Not known	8	(0.3)		
Household Gross Income per Annum				
<£15,000	202	(8.4)	*(8.7)*	-[h]
£15,000-£30,000	577	(24.0)	*(24.9)*	
£30,000-£45,000	539	(22.4)	*(23.3)*	
£45,000-£60,000	401	(16.7)	*(17.3)*	
>£60,000	595	(24.8)	*(25.7)*	
Not known	88	(3.7)		

Tenure				
Own without mortgage	165	(6.9)	*(7.0)*	31%[c]
Own with mortgage	1745	(72.6)	*(73.5)*	40%
Rented from local authority	189	(7.9)	*(8.0)*	19%
Rented privately	275	(11.4)	*(11.6)*	7%
Not known	28	(1.2)		
Number of Bedrooms				
1	35	(1.5)	*(1.5)*	-[i]
2	401	(16.7)	*(16.7)*	
3	1154	(48.0)	*(48.1)*	
4	585	(24.4)	*(24.4)*	
5	166	(6.9)	*(6.9)*	
>5	59	(2.5)	*(2.5)*	
Not known	2	(0.1)		
Number of cars per household				
0	144	(6.0)	*(6.0)*	23%[c]
1	814	(33.9)	*(33.9)*	44%
2	1335	(55.6)	*(55.6)*	27%
>2	107	(4.5)	*(4.5)*	6%
Not known	2	(0.1)		
Age at twins' birth (years)				
Mother	33.6	(5.19)		29.5[a]
Father	36.4	(6.20)		-
BMI (kg/m^2)				
Mother	25.1	(4.82)		26.8[b]
Father	26.4	(3.94)		27.1[b]
Current smoking status of mother				
Yes	306	(12.7)	*(12.8)*	21.0%[b]
No	2094	(87.2)	*(87.2)*	79.0%
Not known	2	(0.1)		
Current smoking status of father				
Yes	466	(19.4)	*(20.5)*	24.0[b]
No	1788	(74.4)	*(78.6)*	66.0
Not known	148	(6.1)		
Smoking status of mother during pregnancy				
Yes	268	(11.2)	*(11.2)*	17%[j]
No	2132	(88.8)	*(88.8)*	83%
Not known	2	(0.1)		
Mother's percent of eating 5 a day[e]				
Yes	790	(32.9)	*(33.2)*	31.0[b]
No	1587	(66.1)	*(66.8)*	69.0
Not known	25	(1.0)		
Father's percent of eating 5 a day[e]				
Yes	663	(27.6)	*(29.3)*	27.0[b]
No	1600	(66.6)	*(70.7)*	73.0
Not Known	139	(5.8)		

[a] Office for National Statistics (2006). ONS Population report for England and Wales. Statistics correspond to parents with life births in 2006

[b] Health Survey for England 2007. (2008). Volume 1. Health lifestyles: knowledge, attitudes and behaviour. Ed R. Craig & N. Shelton. The health and social care Information Centre.

[c] Office for National Statistics (2008). General Household Survey 2007. Data for Great Britain in persons 16 and over.

[d] Italicized percentages are the valid percentages which may be compared to national statistics; non-italicized percentages include missing data. Percentages may not add up to 100 due to rounding.

[e] Consumption of at least 5 portions of fruit and vegetables per day.

[f] Department for Innovation, Universities and Skills. (2008). The level of highest qualification held by adults: England 2007. The education levels published in this report correspond roughly to the categories measured in Gemini, specifically from lowest to highest they include: no qualifications; GCSEs, an Intermediate GNVQ, two AS-levels, NVQs at levels 1 & 2, BTEC general certificates, YT certificates, other RSA certificates or other City and Guilds certificates; 2 A-Levels, 4 AS-Levels, an advanced GNVQ or NVQ level 3; foundation or first degrees, recognized degree-level professional qualifications, NVQ level 4, teaching or nursing qualifications, HE diploma, HNC/HND or equivalent; post-graduate level qualifications and NVQ level 5.

[g] Office for National Statistics (2003). Socio-economic classification of working-age population, summer 2003:Regional Trends 38.

[h] Office for National Statistics (2008). General Household Survey 2007. Data for Great Britain in persons 16 and over. This survey provides information about weekly income that equates to the following figures for annual income which, although not equivocal, provide a useful comparison: 3% < £5200; 8% < £10,400; 7% < £15,600; 7% < £20,800; 8% < £26,000; 17% < £36,400.

[i] There are no national statistics on the actual number of bedrooms per household but the General Household Survey (2007) data for Great Britain in persons 16 and over provides information about number of bedrooms per household that are below standard, at, or above standard.

[j] Infant Feeding Survey 2005 (2007). Early Results: Smoking. The Information Centre for Health and Social Care.

5.3.3. Zygosity questionnaire validation and parental zygosity misclassification

In 100% of the cases tested the questionnaire and DNA-based zygosity test agreed on the classification of twin pairs as MZ and DZ, giving us a great deal of confidence in the zygosity questionnaire. 513 pairs were classified as MZ by both the questionnaire and the parents (MZQ-MZP); 216 pairs were classified as MZ by the questionnaire and as DZ by the parents (MZQ-DZP); 1589 pairs were classified as DZ by both the questionnaire and the parents (DZQ-DZP); only 16 pairs were classified as DZ by the questionnaire and as MZ by the parents (DZQ-MZP). This information will allow me to test whether parental classification of zygosity influenced their scoring of the twins on appetite by comparing twin correlations for MZQ-MZP and MZQ-DZP, and for DZQ-DZP and DZQ-MZP.

5.3.4. Zygosity differences for sample characteristics

There were some differences between MZs and DZs for infant measures. MZs were slightly lighter at birth (mean=2.34 kg, sd=0.55) than DZs (mean=2.52 kg, sd=0.53), [t(4523)=-10.126, p<0.001]; MZs were born slightly earlier (mean=35.6 weeks, sd=2.52) than DZs (mean=36.5 weeks, sd=2.40), [t(2323)=-8.206, p<0.001], and a significantly higher proportion of MZs than DZs (57.1% versus 37.6%) were classified as preterm (χ^2=77.024 (1df), p<0.001). In addition, slightly more MZs than DZs (42.6% versus 36.2%) had reported feeding problems (χ^2=17.231 (1df), p<0.001). However, for all of these differences the effect sizes were considered very small (Cohen's r=0.06 to 0.18).

A few sociodemographic indices differed by zygosity. At the birth of the twins MZ mothers were younger (mean=31.7 years, sd=5.38) than DZ mothers (mean=33.6 years sd=4.95), [t(2326)=-8.344, p<0.001], as were MZ fathers (MZ mean=34.6 years, sd=6.17; DZ mean=36.3, sd=6.17), [t(2181)=-5.802, p<0.001], significantly more MZ families than DZ families (62.7% versus 54.2%) earned less than £45,000 (χ^2=14.037 (1df), p<0.001), and families of MZs had slightly fewer bedrooms (mean=3.2, sd=0.95) compared to DZ families (mean=3.3, sd=0.96) [t(2326)=-8.344, p<0.001] . Again, all of these differences were considered very small (Cohen's r=0.08 to 0.17).

5.3.5 Assumptions for twin models

There was a small but significant correlation between parents for both weight (r=0.23, p<0.001, n=2106) and BMI (r=0.23, p<0.001, n=2106). A slightly (but significantly) higher proportion of MZs were concordant for feeding method than DZs (97.4% versus 92.2%) (χ^2=23.011 (1df), p<0.001), and for feeding problems (90.3% versus 83.8%), (χ^2=17.234 (1df), p<0.001). However, the sizes of the differences were considered very small (Cohen's r=0.1 and 0.09, respectively).

5.3.6 Power

5.3.6.1. Heritability analyses

Gemini is powered at 90% to detect a genetic effect of only 30% with no shared environment effect. With a genetic effect of 25% and a shared environment effect of 5%, the sample has 76% power to detect genetic significance, and 94% power for heritability of 30% with a shared environment effect of 10%. Using a multivariate approach would increase power further.

5.3.6.2. Multiple regression analyses

A model including half of the twins (~2400) would be powered at 99% to detect a small R^2 of 0.02; including only 500 individual twins provides 79% power, and including 600 provides 81% power.

5.4. Discussion

The Gemini cohort will enable me to achieve the objectives of this thesis because it is a large population-based cohort of infant twins. In particular, the sample will allow me to explore individual differences in appetitive traits during the earliest period of life while infants are exclusively fed milk, and the large size of the cohort will ensure that small associations with weight are detected, and that heritability estimates for appetitive traits and weight can be established with some reliability. The questionnaire used to establish the zygosity of the twins performed well, classifying 100% of the twins as MZ or DZ correctly in the random sample that was validated using DNA; this indicates that the MZ and DZ groups can be modelled with confidence in the quantitative genetic analyses. A few differences were identified in the various analyses, none of which were cause for concern. They are discussed below.

There were some differences between the target population and the Gemini cohort. Fewer families with twins born earlier in 2007 took part. However, as ONS contacted families in November 2007 and January 2008, families with twins born between March and April 2007 were the oldest records in the registration data and it may have been the case that these families would have been those who were most likely to have moved address between registering the births and being contacted by ONS. There were not an equal proportion of families from all regions of England and Wales, although there was nevertheless a good distribution which mirrored the population density in general. Although there were slightly fewer mothers in older and younger age groups and slightly more in the 30-34 years group, there were a reasonable proportion of mothers across all age categories.

There also appeared to be some sociodemographic differences between the Gemini sample and national statistics. In-line with many cohort studies, there were slightly more White-British married couples than the population at large. This may partly reflect the fact that the target sample is young parents, whereas national statistics refer to all adults aged 16 and over. Slightly more families owned their property with a mortgage, but fewer owned their property outright, perhaps because families with newborn infants are likely to be younger adults. In general, the Gemini sample was slightly healthier and of a higher social class than the wider population. However, there were a considerable number of families in the different categories of sociodemographic characteristics that were measured ensuring that the cohort includes a good range of families and allowing for these characteristics to be taken into account where appropriate. The twins were comparable to national twin statistics on all tested demographics; nevertheless, twins tend to be born somewhat earlier than singletons (indicated by the mean gestational age) and as a result tend to suffer more postnatal problems including feeding difficulties. Due to the focus of the thesis on appetite it is important to take account of these issues in analyses to allow for generalisation to singletons.

There were some zygosity differences. MZs were born slightly earlier and smaller than DZs in keeping with the literature (Hall, 2003; Hoskins, 1995), and they had a slightly higher incidence of feeding problems, probably as a result of increased prematurity. However the sizes of the effects were very small, suggesting that they would not cause undue influence on analyses. There were also a few sociodemographic differences between MZ and DZ families. MZ mothers and fathers were younger when their infants

were born, they earned slightly less and had slightly fewer bedrooms; these differences may reflect the fact that some of the DZ twins would have been conceived through in vitro fertilisation methods to older and slightly wealthier parents. However, these effects were also very small, not presenting grounds for concern.

There was an indication of a small effect of assortative mating for adiposity because parents were slightly correlated for their weight and BMI (r=0.23), to the extent observed in other studies (r=0.09-0.43) (Allison et al., 1996; Jacobson et al., 2007; Mascie-Taylor, 1987; Silventoinen et al., 2003; Speakman et al., 2007; Tambs et al., 1991), although this correlation could also suggest a shared environment effect. The association was small suggesting that it would not unduly influence heritability analyses, but could serve to reduce heritability estimates for weight very slightly.

There was a small suggestion of an unequal environment for MZ and DZ twins insofar as a slightly higher proportion of MZs were concordant for feeding method than DZs, although this could also suggest an evocative gene-environment correlation if feeding methods are a response to genetically-determined traits such as appetite. In addition, slightly more MZs than DZs were concordant for feeding problems, perhaps as a result of MZs being born smaller and having a higher incidence of feeding problems in general. However, the effect sizes for both of these differences were very small. Lastly, the fact that a substantial proportion of parents misclassified their MZs as DZs will allow me to test if parental classification of twins influences the way they score their appetites, providing a direct test of parental rating biases. This can be tested by comparing twin correlations – e.g. a much higher correlation between MZQ-MZPs than MZQ-DZPs would suggest that parents rate their twins more similarly because they believe them to be identical. However, the very small number of DZQ-MZP pairs will greatly limit conclusions for parents of DZ twins.

Overall, the Gemini cohort provides a large, well-powered and reasonably representative sample to develop an infant appetite questionnaire, to explore associations between appetite and weight, and to explore genetic influences on appetite and weight.

CHAPTER 6. STUDY 1: THE DEVELOPMENT OF THE BABY EATING BEHAVIOUR QUESTIONNAIRE[33]

6.1. Background

The literature review in Chapter 1 identified a cluster of eating behaviours in adults and children that characterise a larger appetite and confer obesity risk, including stronger appetitive responses to food cues, 'valuing' food more highly, lower responsiveness to internal satiety signals and a faster rate of eating. In comparison infant appetite is not well understood and research is needed to identify the feeding behaviours that typify appetite avidity during early life. Nevertheless, the review highlighted that conceptually similar feeding behaviours may be present in infancy, and related to weight. Kron and colleagues (1968) found consistent individual differences in behavioural indicators of sucking avidity during the first four days of life. Using similar behavioural measures, infants at higher familial risk of obesity showed more avid sucking than infants at low risk (Stunkard et al., 2004), and a vigorous feeding style at 2-4 weeks of age was associated with higher adiposity two years later (Agras et al., 1990). Variation in appetitive traits associated with susceptibility to obesity may therefore be present in the first few weeks of life. Infancy is a critical period – rapid weight gain in infancy is associated with later childhood and adult obesity (Baird et al., 2005; Ekelund et al., 2007; Ong, 2006), suggesting that causal processes begin soon after birth; this stresses the importance of identifying candidate mediators such as avid appetitive traits as early as possible to allow intervention before excessive weight gain has occurred.

Chapter 2 also provided some evidence of the heritability of appetitive traits in children and adults. The relative influence of genes and environment on these traits has never been established for the infancy period. Understanding the aetiology of appetite from the

[33] A version of this has been chapter has been written into a paper that is currently under review with Appetite (Llewellyn CH, van Jaarsveld CHM, Johnson J, Carnell S, and Wardle J. Development and factor structure of the Baby Eating Behaviour Questionnaire in the Gemini birth cohort).

beginning of life is important to direct evidence-based intervention work. Large numbers of participants are required to power genetically sensitive designs sufficiently.

Psychometric measures provide one method of quantifying these traits for the purpose of establishing small associations, and obtaining reliable genetic estimates. While behavioural studies provide objectivity and detail, the effort and expense associated with direct observations of feeding behaviour makes it difficult to carry out the large-scale studies that are needed to detect small effects and to give robust estimates of heritability. Psychometric measures which can be completed by parents make it possible to collect appetite data in large samples, and have the additional advantage that parental evaluations aggregate the infant's behaviours over many situations rather than being limited to the single feed usually observed in behavioural studies. Recent work has extended the prospects for carrying out large-scale research into appetite in children with the development of a reliable, valid psychometric measure of children's eating styles, the CEBQ (Carnell & Wardle, 2007). At present, there is not an equivalent measure of appetitive characteristics in infants, preventing large-scale research into these traits in early life.

6.2. Study aim

In response to the need for a similar psychometric measure of infant appetite, this study describes the development of an infant version of the CEBQ that characterises important dimensions of feeding behaviour in the period that infants are still exclusively fed milk.

6.3. Methods

6.3.1. Development of the Baby Eating Behaviour Questionnaire

6.3.1.1. Generation of constructs and items

The appetitive constructs to be included in the Baby Eating Behaviour Questionnaire (BEBQ) were based on: (1) existing scales in the CEBQ that were deemed appropriate for infants in the earliest period of feeding while they are still exclusively fed milk; (2) a review of the literature on milk-feeding, to ascertain if there are distinctive appetitive feeding behaviours related to milk that are only present during early postnatal life; (3) interviews with a sample of mothers with infants aged 6 months or less to establish if the constructs, items and response options generated through (1) and (2) were appropriate.

Seven of the eight CEBQ scales were initially considered appropriate for milk-feeding infants, including 'food responsiveness', 'enjoyment of food', 'emotional overeating', 'desire to drink', satiety responsiveness', 'slowness in eating', and 'emotional under-eating'. 'Food fussiness' by definition must involve the child being weaned so was not selected for the initial stage of the pilot work. The items from the seven selected scales were modified slightly to ensure their suitability for milk-fed infants – e.g. 'My child is always asking for a drink' was adapted to 'My child was always crying for a drink', and 'feeding' or 'mealtimes' were substituted for 'food'. All items were carefully worded to capture appetitive behaviours demonstrated by both breast-fed and bottle-fed infants. Because the period of interest was 0-3 months, but data were collected when infants were older, past tense was used. The same response options as the CEBQ were used: 'never', 'rarely', 'sometimes', 'often', 'always'.

The infant literature indicated that sucking speed (or intensity), responsiveness to food (milk or sweetened solutions) and bottle-emptying characterise appetite avidity in infants and are behaviours that have been associated with higher weight gain or obesity risk (Agras et al., 1990; Li et al., 2008; Millstein, 1980; Stunkard et al., 2004). Rapid feeding

may be a forerunner of faster eating rate in childhood and adulthood, measured in the CEBQ using the 'slowness in eating' scale, while infant-initiated bottle-emptying and heightened sucking avidity in response to sweet solutions are both behaviours indicative of 'food responsiveness', highlighting the importance of these two constructs for this population. An additional item was added to the 'food responsiveness' items that specifically relates to milk-feeding: 'My child frequently wanted more milk than I could provide'. The clinical literature on feeding problems and 'failure to thrive' highlights slow feeding (Reau et al., 1996), low enjoyment of food (Mathisen et al., 1999) and distress during feeding (Chatoor et al., 2001; Mathisen et al., 1999) as some of the features of poor feeding or inadequate appetite. To assess infant distress level we included two other items: 'my baby becomes distressed while feeding', and 'my baby seems contented while feeding'. Two stages of pilot work were conducted to refine the questionnaire items[34].

6.3.1.2. Qualitative pilot work

Cognitive interviewing techniques (Willis, 1999) were used to assess the suitability of the scales and items for measuring the key feeding behaviours and appetitive traits of milk-feeding infants. Two psychologists conducted in-depth qualitative interviews either face-to-face or over the telephone with a convenience sample of 10 mothers who had infants less than 6 months old[35]. The sample was recruited through UCL staff working in the Department of Epidemiology and Public Health and through contacts of the research team.

In-line with Cognitive Theory (Tourangeau, 1984), special attention was paid to the following important cognitive processes involved in psychometric measurement:

(1) Comprehension of the items, such as the question intent and meaning (e.g. "What does the word 'appetite' mean to you?", "Is there a better word to describe it for your baby?", "Is 'feeding' an appropriate word for your baby drinking milk?"), and the appropriateness of the language used (e.g. "Is the word 'child' acceptable to you or would you prefer to use something else?"), which was especially important in ensuring that items elicited similar interpretations for bottle- and breast-feeders.

[34] The full list of the items that were discussed with mothers during the first stage of the pilot work are shown in Appendix 4.1, alongside the original CEBQ items on which they were based for comparison.
[35] I was not involved in the qualitative interviewing, but took sole responsibility for interpreting the findings.

(2) Retrieval from memory of relevant information (e.g. “How did you judge what was a good or poor appetite?”, “Did you compare with friends, or generally feel instinctively about your child’s appetite?”, “Were there any particular behaviours that helped you to decide how your baby was feeling?”), the ease with which mothers could recall the appropriate/ necessary information (e.g. “How well would you say you remember your baby’s feeding behaviour during the first 3 months?”, “Did you find it easy to distinguish these emotions in your child at 3 months of age?”), and the recall strategies used, which was particularly important due to the retrospective nature of the questionnaire (e.g. “Are some aspects of feeding easier to remember than others? Why do you think that is?”).
(3) Decision processes involved in responding to the items such as motivation (i.e. whether the mother was willing to devote sufficient mental effort to answer the question accurately and thoughtfully), and sensitivity and social desirability (e.g. “how comfortable do you feel answering these questions?”).
(4) Response processes to assess whether the mother felt that she could match her internally generated answer to the response categories provided (e.g. “Did you find it easy or difficult to answer the question using the choices we gave you?”, “Would you prefer a different set of options?”, “How are you deciding which answer to give? What are you basing your answer on?”).

Interviews were structured such that general questions were asked at the beginning and at the end of the interview, and in between the separate scales and their items were discussed one-by-one. At the end of each set of items relating to a particular scale mothers were asked if they had any comments or problems; at the end of the interview mothers were asked if they felt there were any feeding behaviours or issues related to feeding that had not been discussed, if they felt that any of the feeding styles were irrelevant (a pertinent question in the light of the fact that the scales were originally designed for much older children), and if they felt that their baby had a consistent feeding style.

Mothers on the whole felt that all feeding behaviours they were aware of were covered by the items. In general, they made judgements about their baby’s appetite instinctively and did not necessarily feel that it was crucial to use other babies as comparators to make inferences about their own. A number of the sample felt that ‘child’ was not an appropriate

description for infants, but also found ‘infant’ too clinical, preferring ‘baby’ to be used in the items instead. The term ‘feeding’ was most appropriate to describe babies’ milk-drinking behaviour, and a ‘feed’ or ‘feeding time’ was a better description of a feeding occasion than a ‘mealtime’. The response options provided suited the mothers, and they found it easy to select an option in response to the items. Mothers felt that they could remember their child’s feeding behaviour vividly during the first 3 months. None of the items were contentious or made the mothers feel uncomfortable.

The ‘slowness in eating’ scale was “easy to answer” – breast-feeding mothers could make judgements based on the sucking sensations, and the average length of a feed; bottle-feeding mothers were able to hear and see sucking, and were aware of the length of time it took their child to finish a bottle of milk; there were large apparent differences in feeding speed even within the small pilot sample. Likewise, there seemed to be a fair amount of variation in how food responsive and satiety responsive mothers felt their infants were. Mothers tended to make judgements about their baby’s level of hunger or feeding demands based upon a number of behavioural indicators including distinctive crying, or reaching for a bottle or for the breast; similarly, pushing the bottle or breast away, or turning the head, indicated satiety. It was suggested that the ‘food responsiveness’ item ‘My child was always crying for a feed’ be modified to a more general item to allow for other indicators of feed demands as well as crying – the suggested revision was ‘my baby was always demanding a feed’. In addition, the item ‘If allowed to my child would feed too much’ was considered vague; it was not clear to mothers if ‘feed too much’ referred to quantity of milk or the frequency of feeds. As most of the other items in the scale referred to feed frequency, this item was made more specific to milk quantity and reworded to ‘If allowed to, my baby would take too much milk’.

One of the ‘satiety responsiveness’ items was also considered too vague – ‘My child cannot take a feed if s/he has had one shortly before’. When mothers were questioned more carefully, an inter-feed interval of about 30 minutes was deemed sufficiently short for their baby to struggle with an additional feed, despite the frequency of feeds in infants this young. Another of the ‘satiety responsiveness’ items (‘My child got full before I finished feeding him/her’) appeared tautological for mothers who were infant-led in their feeding strategies, as they finished feeding when their baby appeared to be full. As an alternative they suggested using an item that captured whether the mother felt that the amount of milk

the baby tended to consume was “about right” or “too little” in her opinion – the item was revised to ‘My baby got full before taking all the milk I thought s/he should have’. The wording of another of the ‘satiety responsiveness’ items was more suitable for breast-feeding mothers than for bottle-feeders – ‘My baby didn’t seem to drink all of the milk I was able to provide’ – and a number of the mothers found the wording cumbersome, and suggested that the item be reworded to capture babies who tend not to be able manage what the mother would regard as a “proper” feed; the item was reworded as ‘My baby found it difficult to manage a complete feed’.

Similarly, the new ‘food responsiveness’ item ‘My child frequently wanted more milk than I could provide’ was deemed as more appropriate for a breast-feeding mother’, although bottle-feeding mothers felt that a small modification to ‘…than I provided’ would rectify the item and breast-feeding mothers were happy with this suggestion. Two of the behaviours captured in the ‘enjoyment of food’ scale proved to be problematic: most mothers felt that it did not make sense to describe their 3-month old baby as ‘looking forward to feeding/ mealtimes’, and others struggled to evaluate whether or not their baby was ‘interested in feeding’ at this young age; some of the mothers found the other two ‘enjoyment of food’ items difficult to distinguish conceptually (‘my child loved feeding’ and ‘my child enjoyed feeding time’) and suggested specifying milk in one item and keeping general feeding occasions in the other.

The two scales relating to emotional eating also proved difficult to answer. In particular, assigning certain feeding behaviours to the baby in circumstances where he or she ‘had nothing else to do’ was not relevant for infants of this age, and some mothers felt the adjectives provided to describe their baby’s affective states were inappropriate for a number of reasons: ‘anxious’ and ‘upset’ felt too cognitive for a young baby; infants are usually ‘grumpy’ or ‘irritable’ when they are hungry and so negative expressions of mood are usually cues that a feed is due, making it difficult to assess if their infant eats more or less in these states. The ‘desire to drink’ scale posed the most serious problem as most infants solely drank milk at 3 months (i.e. no water) so ‘crying for a drink’ was tantamount to crying for a feed; and discerning crying for water or crying for milk was difficult in cases where babies were given both. The other two new items were straight forward to answer for the mothers (‘my child seemed contented while feeding’ and ‘my child became distressed while feeding’).

6.3.1.3. Summary of changes to items and scales based on qualitative pilot work

The findings from the qualitative pilot work were used to develop a pilot questionnaire. 'Child' was changed to 'baby' in all of the items. Both of the 'emotional eating' scales were taken out along with 'desire to drink'; two of the 'enjoyment of food' items were removed ('My child was interested in feeding' and 'My child looked forward to feeding') and one was modified – 'My child loved feeding' became 'My baby loved milk'. Three of the 'satiety responsiveness' items were modified – 'My child could not take a feed if s/he had had one shortly before' specified a time interval and the phrasing was reversed to add more variability to the questions in this scale so that the final item read 'My baby could easily take a feed within 30 minutes of the last one'; 'My baby didn't seem to drink all of the milk I was able to provide' was changed to 'My baby found it difficult to manage a complete feed', and 'My baby got full before I finished feeding him/her' became 'My baby got full before taking all the milk I thought s/he should have'. The 'food responsiveness' item 'My child frequently wanted more milk than I could provide' was modified slightly to 'My baby frequently wanted more milk than I provided', another 'food responsiveness' item 'My baby was always crying for a feed' was modified to 'My baby was always demanding a feed', and a third 'food responsiveness' item 'If allowed to my baby would feed too much' was changed to 'If allowed to my baby would take too much milk'. The same response format was used: 'never', 'rarely', 'sometimes', 'often', 'always' and these responses were scored from 1 ('never') to 5 ('always') for quantitative analyses.

6.3.1.4. Quantitative pilot work

The shortened questionnaire with the four remaining scales ('enjoyment of food', 'food responsiveness', 'satiety responsiveness' and 'slowness in eating') was distributed to a sample of 270 mothers of twin children aged 2-24 months who were contacted via on-line twins clubs (Twinsonline, the Twins Club Forum, and local twins clubs in Kent, London, Cambridge and Swindon)[36]. Questionnaires were either emailed to mothers via the twins clubs organisers or given out to mothers at one of the local meetings. 65 mothers agreed

[36] A list of the 19 items included in the quantitative pilot questionnaire, along with the original CEBQ scales that they were based on are shown in Appendix 4.2.

to take part, and 33 questionnaires were completed and sent back to the research team. The questionnaires were anonymous.

Item means and frequencies were calculated from the pilot sample so that items where 90% or more of the mothers gave the same response could be discarded; inter-item correlations were calculated for each hypothesised scale so that items with very low or extremely high correlations could be discarded. Feedback from mothers or non-response also led to the omission of particular items. Data from the questionnaires were used to inform the final items to be included in the BEBQ. Using these criteria most items performed well, but one was discarded – the 'food responsiveness' item 'Given the choice, my baby would feed most of the time' was very highly correlated with 'If given the chance my baby would always be feeding' and on face validity it measured the same behaviour. The final BEBQ contained 18 items that were intended to measure four appetitive scales: 'enjoyment of food' (4 items), 'food responsiveness' (5 items), 'slowness in eating' (4 items) and 'satiety responsiveness (5 items).

6.3.2. Assessing the factor structure of the BEBQ

The final BEBQ was sent out to the Gemini families in the baseline questionnaire. The data obtained from the Gemini families was analysed to determine the factor structure and reliability of this new questionnaire. As two BEBQs were completed by the parent for the two twins, one was selected at random to be entered in to the analysis in order to adjust for clustering of the twins in families and for rater bias (this was the case with the sub-group analyses as well, described later). The other half of the sample was used as a control and analyses were re-run on them. The findings were the same so only the results from one twin drawn at random are presented. Missing data varied by item (see Table 6.1 for participant numbers of each item).

6.3.2.1. Principal component analysis

There are many different methods for deciphering the underlying structure of a psychometric dataset (Tinsley & Tinsley, 1987). Given the much younger sample (infants

rather than children), the different feeding behaviour (exclusively milk compared with solid food), and the modified items in comparison to the original CEBQ, an exploratory method of analysis was deemed appropriate rather than a confirmatory method to establish whether the items loaded onto the anticipated theoretical scales of the CEBQ, or if the data indicated different latent traits. Two approaches can be used to explore the psychometric properties of a new instrument– factor analysis and principal component analysis (PCA) (Field, 2005). PCA was chosen as it is considered a psychometrically sound procedure and compared to factor analysis it is mathematically less complex (Stevens, 1996).

6.3.2.1.1. Extraction of components

The aim of PCA is to reduce a large number of correlated variables to a few common components that explain the greatest proportion of variance in the data, with the least amount of information lost. This is achieved by calculating common variance shared between variables using a matrix of correlation coefficients. In an iterative process, every possible linear combination of variables is analysed and the combination that leads to the highest proportion of variance explained in the original data is selected. Subsequent components are extracted by the same procedure using remaining variance (Field, 2005). The amount of variance in the data that is explained by each component is denoted by its eigenvalue, the size of which indicates its' substantive importance – the higher the value, the more of the variance is explained by that component. Ultimately, the aim is to select only those components with relatively large eigenvalues and ignore those with smaller values (Field, 2005).

Three methods assist in the selection process. A scree plot presents the eigenvalues of each component graphically in order of magnitude – the point of inflexion may be used to indicate the cut-off point for selecting the most important components (Cattell, 1966). Kaiser (1960) recommends retaining all components with an eigenvalue greater than 1, as this indicates a substantial amount of variance, while Jolliffe (1972; 1986) suggests that compoents with eigenvalues over 0.7 be retained. Field (2005) cautions against component selection based solely upon scree plots, and recommends using Kaiser's criterion with samples >250 and an average communality score $\geq$0.6, or with fewer than 30 items and an average communality score >0.7. For the purposes of analysing the BEBQ,

Kaiser's criterion was used as the sample size was very large (over 2000), only 18 items were included, and the average communalites value was 0.6.

6.3.2.1.2. Choice of rotation method

As most variables tend to load highly onto the most important component, a technique called rotation is used to maximise the loadings of the variables onto individual components and equalise the relative importance of each component, without changing the underlying solution (Field, 2005). Two types of rotation technique may be used: the 'orthogonal' option assumes all of the components are uncorrelated with one another; the 'oblique' method allows components to correlate. An oblique ('direct oblimin') rotation was selected as this method allows inter-correlations among the components that could be related in theoretical terms (Field, 2005), and are correlated in the CEBQ (Mathisen et al., 1999; Wardle et al., 2001b).

An oblique rotation method results in two component matrices – the Pattern matrix which shows the component loadings with the unique contribution of each variable to each component, and the structure matrix which also takes into account the correlations between components when estimating the contribution of each variable to each component. Values in the pattern matrix can be concealed if components are correlated (Graham et al., 2003), so both the structure matrix and the pattern matrices were considered, and both are presented.

6.3.2.1.3. Choice of component-loading value

SPSS was not constrained in terms of the number of components it was asked to generate in order to explore whether the same dimensions that underlie children's appetite in the CEBQ underlie infants' appetite in the BEBQ. Only items with component loadings greater than 0.4 were considered (Stevens, 1996) (i.e. items for which 16% of the variance is explained by the component) in order to ensure only substantial loadings are interpreted. However all items with loadings of 0.1 were generated to understand smaller relationships between the items and other components as well. Horn's parallel analysis (PA) (a Monte-Carlo based simulation method) was used to test whether the eigenvalues obtained for the components identified were statistically significantly higher than the values that would be

expected for components generated through chance, assuming no actual underlying components are present in the dataset (Horn, 1965). This analysis was performed in Monte-Carlo PCA for Parallel Analysis 2.3.

6.3.2.1.4. Missing data

Pairwise deletion of missing cases was used as this method retains all cases with any data and provides a reasonable solution with large datasets and relatively few missing values (Tabachnick & Fidell, 2001). In order to check the appropriateness of this approach missing values were also replaced with imputed scores using the Expectation-Maximisation method[37] and the analysis was rerun on the dataset including imputed scores; the results were the same so only the analysis without imputed data is presented.

6.3.2.1.5. Assumptions

A number of assumptions must be met for PCA to be considered appropriate (Field, 2005):

1. There must be an *adequate sample size*. Empirical research that has experimented with simulated data has indicated that with communalities in the 0.5 range (the value for the BEBQ was found to be 0.6) samples between 100-200 are adequate with relatively few components and indicator variables (MacCallum et al., 1999), suggesting that the sample-size used for the BEBQ (n=2223) is excellent.
2. Every variable should show *high inter-correlations* with some other variables. Some correlations should be $\geq$0.3. Bartlett's test of sphericity is a formal test of this assumption and should be statistically significant. The overall Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy value should be $\geq$0.6, and there are guidelines for different values: 0.5 is 'barely acceptable', 0.5-0.7 is 'mediocre', 0.7-0.8 is 'good', 0.8-0.9 is 'great', and values above 0.9 are 'superb' (Kaiser, 1974). In addition, each item should have a KMO value > 0.5 (variables with values lower than this should be removed).

[37] Expectation-Maximisation (EM) is considered superior to using means; missing scores are imputed using other participant information that predicts observed values in the dataset.

3. Extreme *multicollinearity* or *singularity* should also be avoided. The determinant of the correlation-matrix will indicate this and the value should be greater than 0.00001.
4. *Correlations should be linear*. Spot-checking of scatter-plots of some combinations of variables is adequate to check this (Tabachnick & Fidell, 2001).
5. *The model should fit the data well*. To test the 'goodness of fit' of the model, a correlation matrix is produced that is based on the model rather than the real data (the 'reproduced matrix'), and differences between the matrix based on the model and the matrix based on the observed data indicate the residuals of the model (i.e. differences). Ideally relatively few of these values (less than 50%) should be greater than 0.05. SPSS produces a summary of how many residuals lie above 0.05.
6. The variables should be roughly *normally distributed*, and if they are not transformation of the data should be attempted.

6.3.3. Reliability analysis and summary statistics

The internal reliabilities (or consistencies) of the components derived from the PCA were tested using Cronbach's Alpha (α). Ideally, the α value should be $\geq$0.7, and values lower than this suggest that the scale may be internally unreliable, although greater numbers of items in a scale reduce the alpha value as well (Cortina, 1993).

Two items in the questionnaire with a reversed phrasing ('my baby became distressed while feeding' and 'my baby finished feeding quickly') were reverse-scored for the reliability analysis as inclusion of the actual scores would reduce the α value. Scores were reversed by taking the maximum value of the response scale (5), adding 1 to it (to make it 6) and taking away each participant's actual score from this number (Field, 2005). Individual scores were calculated for each infant for the scales identified through the PCA and reliability analysis by summing all of the available item scores within each scale and dividing the total value by the number of items responded to in that scale. A minimum number of items were required to be completed by participants for a scale score to be calculated (2/3, 3/4, and 4/6).

The normality of the scales was assessed primarily by calculating skewness and kurtosis statistics. Absolute values of less than 1 were deemed 'normal' and values above 1 were deemed non-normal. Means and standard deviation scores as well as medians and interquartile ranges were then calculated for the scales. Pearson's Product Moment correlation coefficient was used to assess bivariate relationships between the normally distributed scales and Spearman's ρ to assess correlations between non-normally distributed scales. For all analyses an alpha level of 0.01 was selected for significance to reduce the risk of a Type 1 error resulting from the large sample and multiple tests.

6.3.4. Sub-group analyses

6.3.4.1. Checking the component structure and scale reliability of sub-groups

Because this was a new questionnaire it was important to check that the same underlying dimensions emerged for different sub-groups, to ensure that it could be used with confidence for all infants. It was particularly important to check that the latent constructs were the same for MZs and DZs in order to ensure that the questionnaire could be used to model the heritability of appetite[38]; and it was also checked that males and females showed the same patterns.

In addition, it was hypothesised that any factor that potentially influenced the infant's feeding style or appetite may influence the underlying structure of the questionnaire. The coordination of sucking, swallowing and breathing necessary for normal oral feeding is generally not established until 34 weeks postconceptional age (Wolff, 1968). Infants who are born before this gestational milestone are therefore likely to have been tube fed and may have experienced problems establishing normal oral feeding – this could have affected the way parents scored their appetite using the BEBQ, and potentially the resulting component structure. Gestational age in weeks was used to group the infants into those born before 34 weeks gestation and those born at or after 34 weeks gestation.

[38] A key assumption is that the researcher is modeling the same construct in MZs and DZs, or the heritability analysis is not valid.

Infants with reported feeding problems may also have been scored differently compared to those with no reported feeding problem at any time, and so the underlying structure could differ for these groups also. Lastly, because breast-feeding and bottle-feeding are different behaviours, it was also possible that the latent appetitive dimensions differed by feeding mode. Responses were categorised into 'breast-fed' or 'bottle-fed' according to the dominant behaviour in order to maximise the numbers of infants in each group for the PCA: 'breast-fed' included 'entirely' or 'mostly' breast-fed infants; 'bottle-fed included 'mostly', 'almost entirely' or 'entirely' bottle-fed infants.

The PCA and reliability analyses were therefore re-run on 10 sub-groups to check if the findings were the same: (1) MZs; (2) DZs; (3) males; (4) females; (5) infants born before 34 weeks gestation; (6) infants born at or after 34 weeks gestation; (7) infants with no reported feeding problems[39]; (8) infants with reported feeding problems; (9) infants who were mainly bottle-fed; (10) infants who were mainly breast-fed. SPSS was not constrained in the number of components it was asked to generate in order to explore if the same components emerged from the data without manipulation.

6.3.4.2. Mean differences by sub-group

I was also interested to find out if any of the factors described above influenced the mean scores for the BEBQ scales, and to assess the effect of other potential influences on the BEBQ scores. In order to explore differences in feeding method, infants who were equally bottle- and breast-fed were also included to maximize information, so that three groups were examined ('bottle-fed', 'breast-fed' and 'mixed-fed'). Age of the infants at BEBQ completion was tested because of the retrospective nature of the questionnaire – it was important to check that mothers who responded when their infants were older were not biased by their infants having a larger appetite as a result of being bigger.

Birth weight was of interest because it would be expected that to some extent infants who are born bigger have larger appetites in order to support their additional energy requirements. Smoking during pregnancy ('yes' or 'no') was of interest as well because of

[39] 'No feeding problems' included infants with no reported feeding problem at any time; 'feeding problems' included all infants with any reported feeding problem at any time.

the association that has sometimes found between maternal smoking during pregnancy, lighter birth weight, and subsequent obesity risk (Wideroe et al., 2003; von Kries.R et al., 2002; Vik et al., 1996; Toschke et al., 2002; Power & Jefferis, 2002; Al Mamun et al., 2006). It is possible that obesity risk is mediated through foetal 'progamming' (i.e. upregulation) of appetitive pathways; this may be occurring in response to restricted growth which results in part from reduced blood flow causing 'under-nutrition' (Pringle et al., 2005), or through the effects of substances in the cigarettes such as nicotine, which has been shown to programme appetitive pathways in animal studies (Kane et al., 2000; Li et al., 2000).

Family social class was also of interest because lower SES has sometimes been associated with higher weight in early to middle childhood and a greater risk of obesity (Dubois & Girard, 2006; Langnase et al., 2002; Semmler et al., 2009; Stamatakis et al., 2010a). A range of indicators of socio-economic class were measured in Gemini from which to choose, including maternal and paternal education, maternal and paternal occupation as the National Statistics Socio-economic Classification (NSSEC), household gross annual income, number of cars, housing tenure and number of bedrooms. Two indices of SES were chosen – maternal education and NSSEC. Maternal education was selected because it is considered by researchers in the field of SES to be of particular relevance for health behaviours, a reliable marker of nutritional knowledge which may relate to feeding behaviour, and has been associated with feeding style in children (Winkleby et al., 1992; Pill et al., 1995; Wardle et al., 2002; Popkin et al., 2003; Saxton et al., 2009). To aid interpretation of the sub-group analyses in this chapter it was collapsed into three groups, 'low', 'intermediate' and 'high' (as described in Chapter 5, section 5.2.2.1.3.3).

NS-SEC was included as well for a number of reasons – it is now used in all official statistics and surveys in the UK relating to health research (Galobardes et al., 2006) allowing for comparisons with other studies; it takes into account both the maternal and paternal occupations while maternal education excludes paternal factors; it is reasonably inclusive of a number of factors relating to SES including income, financial security and psychosocial processes relating to health behaviours; it has been related to a variety of health outcomes (Bartley, 2004). These two measures correlated only 0.45 in the sample, indicating that they measure different dimensions of social class, so including both would

provide additional information. Lastly, it was important to check if appetite scores differed between non White-British and White-British infants for future heritability analyses because heritability estimates are highly population-dependent.

I used independent samples t-tests and analyses of variance (ANOVAs) to assess two-level and three-level group differences across all of the scales[40]. For three-level group variables that were found to have significant differences in the ANOVAs, pairwise post-hoc comparisons were carried out to explore which groups were significantly different from one another using the Bonferroni correction for multiple comparisons (an alpha-level of <0.01 was adopted to account for the large sample size). Cohen's d was calculated for significant differences to indicate the size of the effect across groups (including significant pairwise differences within three-level groups). Cohen provided categories for effect sizes based on their magnitude: 'small', 0.2 to 0.3; 'medium', ~0.5; 'large', 0.8 to ∞ (Cohen, 1988). Pearson's Product Moment correlation coefficient was used to assess associations between birth weight and normally distributed BEBQ scale scores, and Spearman's ρ to assess the correlation between birth weight and non-normally distributed scales. Spearman's rho was used to explore associations between the age of the infants when the parents completed the BEBQ and the scale scores, because age at questionnaire completion was positively skewed; Spearman's rho was also used to evaluate whether gestational age as a continuous measure was related to BEBQ scale scores, as gestational age was negatively skewed.

6.4. Results

6.4.1. Data screening

The value of the determinant of the correlation-matrix was >0.00001 (0.001) and no variables in the correlation matrix correlated too highly (all <0.7) confirming that multicollinearity or singularity was not a problem, and at the same time Bartlett's Test of Sphericity was significant indicating the 'factorability' of the dataset (i.e. all variables

[40] I also checked for group differences in 'enjoyment of food' using the non-parametric equivalents (Mann-Whitney U test and Kruskal-Wallis test) as this scale was not normally distributed. The results were the same so only the results from the t-tests and ANOVAs are reported.

correlate fairly well with all others). The Kaiser-Meyer-Olkin measure of sampling adequacy (KMO) was 0.866 which is classified as 'great' (Kaiser, 1974) and the KMO value for every individual variable was greater than 0.8 confirming that PCA is appropriate for the data. Less than 50% (35%) of the reproduced residuals had absolute values greater than 0.05 indicating that the model fitted the data well.

The descriptive statistics for each of the BEBQ items, including skewness and kurtosis statistics, are shown in Table 6.1. All of the 'enjoyment of food' items were skewed – 'My baby seems contented while feeding', 'My baby loved milk' and 'My baby enjoyed feeding time' were negatively skewed indicating that the majority of babies appear to be contented while being fed and enjoy milk and feeding occasions, while 'My baby became distressed while feeding' was positively skewed indicating that it is unusual for babies to appear to be distressed by feeding. In addition, one of the 'food responsiveness' items ('If given the chance my baby would always be feeding') and one of the 'satiety responsiveness' items ('My baby could easily take a feed within 30 minutes of the last one') were also positively skewed, indicating that on the whole, most babies would not always be feeding if allowed to, and that most babies are unable to manage a feed shortly after a previous feed. There was substantial leptokurtic kurtosis for 3 of the 'enjoyment of food' items ('My baby seems contented while feeding', 'My baby loved milk', 'My baby enjoyed feeding time'), and one 'satiety responsiveness' item ('My baby could easily take a feed within 30 minutes of the last one') highlighting that the frequency distributions had high peaks and were thin in the tails, indicated by positive kurtosis values.

Two approaches were considered to rectify the variables that were not normally distributed. One option would be to dichotomise the variables, but this leads to an unacceptable loss of information in the data. The second option would be to transform the variables to create approximately normally distributed data. However, only a few items were non-normally distributed, but transformation of all items is required and this resulted in disruption to the normality of the other items; secondly, nine transformations were attempted (cubic, square, identity, square root, log, 1/square root, inverse, 1/square, 1/cubic) and did not succeed in normalising any of the problematic variables[41]. For this reason all items were entered in the PCA without transformation.

[41] To illustrate this Appendix 4.3 shows the unsuccessful transformations for one of the problematic items - 'My baby seems contented while feeding'.

Table 6.1. Descriptive statistics for each item of the BEBQ

Item*	*n***	mean (sd)	skewness (se)	kurtosis (se)
My baby seemed contented while feeding	2350	4.29 (0.84)	-1.36 (0.05)	2.18 (0.10)
My baby enjoyed feeding time	2370	4.22 (0.83)	-1.18 (0.05)	1.77 (0.10)
My baby loved milk	2345	4.46 (0.83)	-1.67 (0.05)	2.70 (0.10)
My baby became distressed while feeding (R)	2368	4.14 (0.96)	-1.06 (0.05)	0.65 (0.10)
If given the chance my baby would always be feeding	2360	1.83 (0.98)	1.17 (0.05)	0.93 (0.10)
Even when my baby had just eaten well s/he was happy to feed again if offered	2348	1.89 (0.92)	0.89 (0.05)	0.33 (0.10)
My baby could easily take a feed within 30 minutes of the last one	2342	1.75 (0.94)	1.22 (0.05)	1.04 (0.10)
My baby was always demanding a feed	2360	2.19 (0.96)	0.58 (0.05)	-0.04 (0.10)
If allowed to my baby would take too much milk	2358	1.97 (1.05)	0.96 (0.05)	0.29 (0.10)
My baby frequently wanted more milk than I provided	2322	2.08 (0.95)	0.89 (0.05)	0.73 (0.10)
My baby had a big appetite	2373	3.29 (1.06)	-0.12 (0.05)	-0.55 (0.10)
My baby fed slowly	2370	2.69 (1.15)	0.31 (0.05)	-0.65 (0.10)
My baby finished feeding quickly (R)	2375	3.05 (1.07)	-0.14 (0.05)	-0.58 (0.10)
My baby took more than 30 minutes to finish feeding	2372	2.46 (1.23)	0.43 (0.05)	-0.80 (0.10)
My baby sucked more and more slowly during the course of a feed	2354	2.68 (1.03)	0.12 (0.05)	-0.60 (0.10)
My baby got full up easily	2363	2.59 (1.01)	0.28 (0.05)	-0.51 (0.10)
My baby got full before taking all the milk I thought s/he should have	2364	2.37 (0.99)	0.37 (0.05)	-0.38 (0.10)
My baby found it difficult to manage a complete feed	2358	2.35 (1.01)	0.49 (0.05)	-0.26 (0.10)

*Items marked with (R) have been reversed for scoring purposes.
**The *n* is based on half the sample of twins drawn from each family at random and pairwise deletion of cases.

6.4.2. Principal components analysis

6.4.2.1. Component structure of the full BEBQ

The PCA revealed four components within the dataset with eigenvalues of 3.618 (Component 1), 3.514 (Component 2), 3.302 (Component 3) and 2.717 (Component 4), using Kaiser's criterion of selecting components with Eigenvalues over 1.0 prior to rotation. Parallel Analysis confirmed that the eigenvalues were above the values that would be expected for components generated through chance. The four components explained 59.7% of the variance in the 18 items[42].

The same four components emerged in the Structure and the Pattern matrices (shown in Tables 6.2 and 6.3 respectively), in that the same variables comprised the four components. Inspection of the components indicated four distinct appetitive traits which matched closely the CEBQ structure. Component 1 included the four items developed to measure 'enjoyment of food'. Component 2 contained all the items originally developed to measure 'food responsiveness' and two items that were originally designed to measure 'satiety responsiveness' – 'my baby could easily take a feed within 30 minutes of the last one' and 'my baby had a big appetite'. In infants, 'my baby could easily take a feed within 30 minutes of the last one' may be more indicative of responsiveness to cues of feeding as it did not load on to the 'satiety responsiveness' component at all. Although 'My baby had a big appetite' loaded most strongly on to 'food responsiveness' (it loads most strongly on to 'satiety responsiveness' in the CEBQ) it also loaded above 0.4 on to the other three components in the Structure matrix (which allows inter-correlations between the components), and at face-value it measures overall appetite. Because this item did not sit clearly on any one component the decision was made to use it as an individual item to measure overall 'appetite size' rather then 'food responsiveness', as the scales are designed to measure four distinct appetitive traits. The PCA was re-run without this item to confirm that the component structure remained the same. Component 3 contained all the items designed to measure 'slowness in eating' and Component 4 consisted of three of the five items that were designed to measure 'satiety responsiveness'.

[42] It is not possible to estimate the unique variance explained by each factor if an oblique rotation is used as components are correlated.

Table 6.2. Component loadings for all items of the Baby Eating Behaviour Questionnaire (Structure Matrix)

Item[a]	*n*[b]	Original Scale[c]	Components Determined Through PCA[d]			
			1 (EF)	2 (FR)	3 (SE)	4 (SR)
My baby seemed contented while feeding	2350	EF	**0.84**	-	-0.28	-0.16
My baby enjoyed feeding time	2370	EF	**0.82**	0.17	-0.30	-0.25
My baby loved milk	2345	EF	**0.76**	0.22	-0.32	-0.39
My baby became distressed while feeding (R)	2368	EF	**0.75**	-0.11	-0.26	-0.22
If given the chance my baby would always be feeding	2360	FR	-	**0.80**	-	-
Even when my baby had just eaten well s/he was happy to feed again if offered	2348	FR	0.11	**0.78**	-	-0.16
My baby could easily take a feed within 30 minutes of the last one	2342	SR	0.11	**0.73**	-	-
My baby was always demanding a feed	2360	FR	-	**0.64**	-	0.13
If allowed to my baby would take too much milk	2358	FR	-	**0.62**	-0.25	-0.19
My baby frequently wanted more milk than I provided	2322	FR	-	**0.57**	-0.21	-0.24
My baby had a big appetite	2373	SR	**0.41**	**0.55**	**-0.43**	**-0.43**
My baby fed slowly	2370	SE	**-0.41**	-0.14	**0.86**	0.37
My baby finished feeding quickly (R)	2375	SE	-0.27	-0.30	**0.82**	0.18
My baby took more than 30 minutes to finish feeding	2372	SE	-0.31	-	**0.79**	0.20
My baby sucked more and more slowly during the course of a feed	2354	SE	-0.16	0.14	**0.50**	**0.42**
My baby got full up easily	2363	SR	-0.16	-	0.13	**0.79**
My baby got full before taking all the milk I thought s/he should have	2364	SR	**-0.42**	-0.20	0.39	**0.77**
My baby found it difficult to manage a complete feed	2358	SR	**-0.58**	-0.25	**0.47**	**0.70**

[a] Items marked with (R) have been reversed for scoring purposes.
[b] The *n* is based on half the sample of twins drawn from each family at random and pairwise deletion of cases.
[c] Original Child Eating Behaviour Questionnaire scales upon which the items were based. Abbreviations: EF, 'enjoyment of food'; FR, 'food responsiveness'; SE, 'slowness in eating'; SR, 'satiety responsiveness'.
[d] Only component loadings over 0.1 are presented. Component loadings over 0.4 are bolded.

Table 6.3. Component loadings for all items of the Baby Eating Behaviour Questionnaire (Pattern Matrix)

Item[a]	*n*[b]	Original Scale[c]	Components Determined Through PCA[d]			
			1 (EF)	2 (FR)	3 (SE)	4 (SR)
My baby seemed contented while feeding	2350	EF	**0.86**	-	-	-
My baby enjoyed feeding time	2370	EF	**0.81**	-	-	-
My baby became distressed while feeding (R)	2345	EF	**0.75**	-0.19	-	-
My baby loved milk	2368	EF	**0.70**	0.13	-	-0.18
If given the chance my baby would always be feeding	2360	FR	-	**0.81**	0.14	-
Even when my baby had just eaten well s/he was happy to feed again if offered	2348	FR	-	**0.77**	-	-
My baby could easily take a feed within 30 minutes of the last one	2342	SR	-	**0.74**	-	-
My baby was always demanding a feed	2360	FR	-	**0.67**	-	0.18
If allowed to my baby would take too much milk	2358	FR	-0.11	**0.60**	-0.20	-
My baby frequently wanted more milk than I provided	2322	FR	-0.10	**0.55**	-0.14	-0.16
My baby had a big appetite	2373	SR	0.22	**0.48**	-0.23	-0.24
My baby finished feeding quickly (R)	2370	SE	**-**	-0.21	**0.84**	-0.14
My baby took more than 30 minutes to finish feeding	2375	SE	**-**	0.12	**0.80**	-
My baby fed slowly	2372	SE	-0.12	-	**0.79**	-
My baby sucked more and more slowly during the course of a feed	2354	SE	-	0.22	**0.43**	0.32
My baby got full up easily	2363	SR	-	-	-0.14	**0.85**
My baby got full before taking all the milk I thought s/he should have	2364	SR	-0.19	-	**-**	**0.67**
My baby found it difficult to manage a complete feed	2358	SR	-0.36	-0.13	0.16	**0.53**

[a] Items marked with (R) have been reversed for scoring purposes.
[b] The *n* is based on half the sample of twins drawn from each family at random and pairwise deletion of cases.
[c] Original Child Eating Behaviour Questionnaire scales upon which the items were based. Abbreviations: EF, 'enjoyment of food'; FR, 'food responsiveness'; SE, 'slowness in eating'; SR, 'satiety responsiveness'.
[d] Only component loadings over 0.1 are presented. Component loadings over 0.4 are bolded.

6.4.2.2. Re-testing of assumptions and sampling adequacy of the BEBQ following exclusion of 'my baby has a big appetite'

Assumptions were rechecked following exclusion of 'My baby had a big appetite'. All of the statistics indicated that the PCA was appropriate for the reduced 17-item dataset. The value of the determinant of the correlation-matrix was >0.00001 (0.001), no variables in the correlation matrix correlated too highly (all <0.7), and Bartlett's Test of Sphericity was significant. The Kaiser-Meyer-Olkin measure of sampling adequacy (KMO) was 0.853 and individual KMO values were all greater than 0.8. Less than 50% (34%) of the reproduced residuals had absolute values greater than 0.05. Furthermore the average of the communalities remained at 0.6 confirming that Kaiser's Criterion was still a suitable indicator of the number of components to extract.

6.4.2.3. Component structure of the shorter BEBQ

The PCA revealed the same component structure for the BEBQ after the general appetite question had been removed from the dataset. The same items comprised Component 1 ('enjoyment of food'), Component 2 ('food responsiveness'), Component 3 ('slowness in eating') and Component 4 ('satiety responsiveness') in both the Structure and Pattern matrices (Tables 6.4 and 6.5 respectively), and component loadings for all items were virtually the same as the loadings from the first analysis that included 18 items.

Table 6.4. Component loadings for the Baby Eating Behaviour Questionnaire excluding the item 'My baby had a big appetite' (Structure Matrix)

Item[a]	*n*[b]	Original Scale[c]	Components Determined Through PCA[d]			
			1 (EF)	2 (FR)	3 (SE)	4 (SR)
My baby seemed contented while feeding	2350	EF	**0.84**	-	-0.28	-0.15
My baby enjoyed feeding time	2370	EF	**0.82**	0.16	-0.30	-0.24
My baby loved milk	2368	EF	**0.76**	0.20	-0.31	-0.38
My baby became distressed while feeding (R)	2345	EF	**0.75**	-0.12	-0.26	-0.22
If given the chance my baby would always be feeding	2360	FR	-	**0.81**	-	-
Even when my baby had just eaten well s/he was happy to feed again if offered	2348	FR	0.11	**0.79**	-	-0.16
My baby could easily take a feed within 30 minutes of the last one	2342	SR	0.11	**0.74**	-	-
My baby was always demanding a feed	2360	FR	-	**0.64**	-	0.13
If allowed to my baby would take too much milk	2358	FR	-	**0.62**	-0.25	-0.18
My baby frequently wanted more milk than I provided	2322	FR	-	**0.57**	-0.21	-0.23
My baby fed slowly	2372	SE	**-0.40**	-0.13	**0.86**	0.37
My baby finished feeding quickly (R)	2370	SE	-0.27	-0.25	**0.82**	0.15
My baby took more than 30 minutes to finish feeding	2375	SE	-0.31	-	**0.79**	0.20
My baby sucked more and more slowly during the course of a feed	2354	SE	-0.16	0.14	**0.51**	**0.43**
My baby got full up easily	2363	SR	-0.16	-	0.13	**0.80**
My baby got full before taking all the milk I thought s/he should have	2364	SR	**-0.42**	-0.19	0.39	**0.77**
My baby found it difficult to manage a complete feed	2358	SR	**-0.57**	-0.22	**0.46**	**0.69**

[a] Items marked with (R) have been reversed for scoring purposes.
[b] The *n* is based on half the sample of twins drawn from each family at random and pairwise deletion of cases.
[c] Original Child Eating Behaviour Questionnaire scales upon which the items were based. Abbreviations: EF, 'enjoyment of food'; FR, 'food responsiveness'; SE, 'slowness in eating'; SR, 'satiety responsiveness'.
[d] Only component loadings over 0.1 are presented. Component loadings over 0.4 are bolded.

Table 6.5. Component loadings for the Baby Eating Behaviour Questionnaire excluding the item 'My baby had a big appetite' (Pattern Matrix)

Item[a]	*n*[b]	Original Scale[c]	Components Determined Through PCA[d]			
			1 (EF)	2 (FR)	3 (SE)	4 (SR)
My baby seemed contented while feeding	2350	EF	**0.86**	-	-	-
My baby enjoyed feeding time	2370	EF	**0.81**	-	-	-
My baby became distressed while feeding (R)	2368	EF	**0.75**	-0.18	-	-
My baby loved milk	2345	EF	**0.70**	0.13	-	-0.18
If given the chance my baby would always be feeding	2360	FR	-	**0.82**	0.13	-
Even when my baby had just eaten well s/he was happy to feed again if offered	2348	FR	-	**0.78**	-	-
My baby could easily take a feed within 30 minutes of the last one	2342	SR	-	**0.75**	-	-
My baby was always demanding a feed	2360	FR	-	**0.67**	-	0.17
If allowed to my baby would take too much milk	2358	FR	-	**0.60**	-0.20	-
My baby frequently wanted more milk than I provided	2322	FR	-	**0.55**	-0.14	-0.16
My baby finished feeding quickly (R)	2375	SE	**-**	-0.20	**0.84**	-0.14
My baby took more than 30 minutes to finish feeding	2372	SE	**-**	0.11	**0.80**	-
My baby fed slowly	2370	SE	-0.13	-	**0.79**	-
My baby sucked more and more slowly during the course of a feed	2354	SE	-	0.20	**0.44**	0.32
My baby got full up easily	2363	SR	-	-	-0.13	**0.85**
My baby got full before taking all the milk I thought s/he should have	2364	SR	-0.20	-0.10	**-**	**0.67**
My baby found it difficult to manage a complete feed	2358	SR	-0.37	-0.13	0.16	**0.53**

[a] Items marked with (R) have been reversed for scoring purposes.
[b] The *n* is based on half the sample of twins drawn from each family at random and pairwise deletion of cases.
[c] Original Child Eating Behaviour Questionnaire scales upon which the items were based. Abbreviations: EF, 'enjoyment of food'; FR, 'food responsiveness'; SE, 'slowness in eating'; SR, 'satiety responsiveness'.
[d] Only component loadings over 0.1 are presented. Component loadings over 0.4 are bolded.

6.4.3. Reliability analyses and descriptive statistics

Cronbach's alphas were calculated for the four subscales identified in the PCA, excluding 'My baby had a big appetite'. All scales were internally reliable: 'enjoyment of food', α=0.81; 'food responsiveness', α=0.79; 'slowness in eating', α=0.76; 'satiety responsiveness', α=0.73. 'Enjoyment of food' was negatively skewed (with a skewness statistic lower than -1.0) indicating that the majority of infants enjoyed milk and feeding times; there was also substantial leptokurtic kurtosis for this scale highlighting that the peak was high and it was thin in the tails (a kurtosis statistic greater than 1.0). The other scales were reasonably normally distributed[43]. The summary statistics for the four scales and 'appetite size' are shown in Table 6.6.

Table 6.6. Descriptive statistics for the Baby Eating Behaviour Questionnaire

Baby Eating Behaviour Questionnaire Scale	mean	sd	Median	IQR	*n*
'Enjoyment of food'	4.28	0.70	4.50	0.75	2355
'Food responsiveness'	1.95	0.68	1.83	0.83	2360
'Slowness in eating'	2.72	0.86	2.75	1.25	2372
'Satiety responsiveness'	2.44	0.81	2.33	1.00	2367
'Appetite size'	3.29	1.06	3.0	1.00	2373

In-line with expectations, the four components were correlated (Table 6.7.). 'Enjoyment of food' was modestly and negatively correlated with 'slowness in eating' (-0.36) and 'satiety responsiveness' (-0.45) indicating that babies who enjoy their food also tend to feed more rapidly, and be less sensitive to internal cues of satiety. Likewise 'food responsiveness' was negatively correlated with 'slowness in eating' (-0.10) and 'satiety responsiveness' (-0.21), although the size of the correlations were smaller than those between 'enjoyment of food', 'satiety responsiveness' and 'slowness in eating', suggesting that babies who are more responsive to cues of feeding also tend to feed slightly more rapidly and are a little

[43] The skewness and kurtosis statistics for the four BEBQ scales and 'appetite size' are shown in Appendix 4.4.

less satiety responsive. 'Slowness in eating' and 'satiety responsiveness' were modestly interrelated (0.45), indicating that rapid feeding and low satiety sensitivity are behaviours that tend to be observed together. The single item 'my baby had a big appetite' was positively correlated with both 'enjoyment of food' (0.34) and 'food responsiveness' (0.46) and negatively correlated with 'slowness in eating' (-0.37) and 'satiety responsiveness' (-0.48) indicating that this item is a good indicator of overall appetite avidity.

Table 6.7. Pairwise correlation matrix showing the inter-relationships between the scales of the Baby Eating Behaviour Questionnaire

BEBQ Scale	Correlation coefficient (*n*) [a]			
	'Enjoyment of food'	**'Food responsiveness'**	**'Slowness in eating'**	**'Satiety Responsiveness'**
'Food responsiveness'	0.04 (2340)	-	-	
'Slowness in eating'	**-0.36**[b] (2351)	**-0.10**[b] (2358)	-	
'Satiety responsiveness'	**-0.45**[b] (2346)	**-0.21**[b] (2354)	**0.45**[b] (2366)	
'Appetite size'	**0.34**[b] (2349)	**0.46**[b] (2353)	**-0.37**[b] (2366)	**-0.48**[b] (2362)

[a] Spearman's rho was used for correlations with 'enjoyment of food'; Pearson's product moment correlation coefficient was used for correlations between normally distributed scales ('food responsiveness', 'slowness in eating', 'satiety responsiveness', 'appetite size').
[b] Correlations significant at an alpha level of <0.001 (bolded).

6.4.4. Sub-group analyses

6.4.4.1. Component structure and reliability of the BEBQ for sub-groups

The PCA was repeated for the 10 subgroups to ascertain if the component structure of the full 18-item BEBQ differed by zygosity, sex, gestational age (<34 weeks or ≥34 weeks), feeding problems ('yes' or 'no') or feeding method ('bottle' or 'breast'). The same component structure was identified for all 10 sub-groups, despite not instructing SPSS to generate 4 components in any case. Moreover, for every group the stand alone item 'my baby had a big appetite' loaded on to the four scales with component loadings of 0.3 or higher, including smaller sub-groups where component loadings would be expected to be

much less reliable. The PCA was then repeated for the 10 subgroups on the 17 items, excluding 'My baby has a big appetite' and the same component structure was again reproduced for each group, confirming that four components underlie infant appetite, even after taking into account sample differences. The Cronbach's alphas for each group were similar to the values for the whole sample (Table 6.8), indicating that the scales had good internal reliability for all the groups.

Table 6.8. Cronbach's alphas for the whole sample and sub-groups

Sample	Cronbach's alpha (*n*)			
	'Enjoyment of Food'	'Food Responsiveness'	'Slowness in Eating'	'Satiety Responsiveness'
Whole sample	**0.81** (2319)	**0.79** (2263)	**0.76** (2346)	**0.73** (2342)
Monozygotic twins	**0.84** (702)	**0.80** (687)	**0.75** (711)	**0.70** (714)
Dizygotic twins	**0.80** (1553)	**0.79** (1517)	**0.76** (1571)	**0.73** (1564)
Males	**0.82** (1150)	**0.80** (1129)	**0.75** (1164)	**0.71** (1165)
Females	**0.81** (1169)	**0.78** (1134)	**0.76** (1182)	**0.73** (1177)
No feeding problems	**0.76** (1412)	**0.79** (1381)	**0.73** (1430)	**0.70** (1422)
Feeding problems	**0.85** (906)	**0.80** (881)	**0.78** (915)	**0.74** (919)
Born ≥34 weeks	**0.81** (2004)	**0.79** (1954)	**0.74** (2025)	**0.72** (2022)
Born <34 weeks	**0.84** (305)	**0.79** (299)	**0.81** (311)	**0.72** (310)
Bottle-fed	**0.82** (1237)	**0.80** (1206)	**0.78** (1254)	**0.74** (1258)
Breast-fed	**0.79** (771)	**0.78** (751)	**0.72** (776)	**0.67** (770)

6.4.4.2. Mean differences and associations

The means and standard deviations for the different groups are shown Table 6.9. There was a small but significant effect of gestational age on BEBQ scores such that infants born before 34 weeks postconceptional age were rated as enjoying feeding to a lesser extent [$t(385.783)=-2.795$, $p=0.005$], being less food responsive [$t(2348)=-4.969$, $p<0.001$], being

more sensitive to internal cues of satiety [t(2355)=4.542, p<0.001], feeding more slowly [t(385.406)=5.300, p<0.001], and having a lower overall appetite [t(2361)=-6.841, p<0.001] compared with infants born at or after 34 weeks gestation. The effect of gestational age was medium for 'slowness in eating' (d=0.54) and small for the other traits (d=0.19-0.29) [44]. The findings were the same treating gestational age as a continuous measure – more premature infants were reported as enjoying food less (ρ=0.06, p=0.005, n=2345), being less responsive to food (ρ=0.13, p< 0.001, n=2350), feeding more slowly (ρ=-0.11, p< 0.001, n=2362), being more sensitive to internal cues of satiety (ρ=-0.12, p< 0.001, n=2357), and having a smaller overall appetite (ρ=0.15, p< 0.001, n=2363) than infants born at a later gestational age, although the effect appeared to be small in each case.

The same differences were found between infants with reported feeding problems and infants with no reported feeding problems. 'Problem-feeders' were rated as enjoying food less [t(1574.024)=8.927, p<0.001], being less responsive to food [t(2357)=2.661, p=0.008], being more satiety sensitive [t(1830.658)=-8.324, p<0.001], feeding more slowly [t(1840.982)=-7.885, p<0.001], and having a lower overall appetite [t(2370)=5.577, p<0.001] compared with infants with no feeding problems at all. The size of the effect on each scale was small to medium (d=0.11-0.45), the largest effect being observed for 'enjoyment of food' (d=0.45) and the smallest for 'food responsiveness' (d=0.11).

There was a small but significant effect of birth weight on some appetitive traits. Infants who were born bigger enjoyed food to a greater extent (ρ=0.11, p< 0.001, n=2289), fed at a faster rate (r=-0.12, p<0.001, n=2306), were less sensitive to internal satiety cues (r=-0.16, p<0.001, n=2302) and had a larger overall appetite (r=0.16, p<0.001, n=2308) than infants born smaller. Birth weight was not significantly associated with 'food responsiveness' (r=0.05, p=0.019, n=2294).

There were some differences in scores by feeding method for 'food responsiveness' [F(2,2262)=45.079, p<0.001], 'satiety responsiveness' [F(2,2268)=23.205, p<0.001], and 'appetite size' [F(2,2274)=16.659, p<0.001]. Breast-fed babies were rated as being more responsive to food than either mixed-fed (p<0.001] or bottle-fed babies (p<0.001), while mixed-fed and bottle-fed babies were not different from one another (p=0.210). The same

[44] The categories provided by Cohen for d are: small, 0.2 to 0.3; medium, ~0.5; large, 0.8 to ∞ (Cohen, 1988).

pattern was observed for 'satiety responsiveness' with breast-fed babies being scored lower on 'satiety sensitivity' than either mixed-fed (p<0.001) or bottle-fed infants (p<0.001), but no difference was found for this characteristic between mixed-fed and bottle-fed infants (p=1.00). Breast-fed infants also had significantly higher scores for 'appetite size' than bottle-fed infants (p<0.001), but mixed-fed infants did not differ from either breast-fed (p=0.109) or bottle-fed babies (p=0.464). In each case the size of the difference was small to medium (d=0.26-0.43).

Male infants scored significantly higher on 'food responsiveness' [t(2339.535)=3.621, p<0.001] and 'appetite size' [t(2363.694)=5.309, p<0.001], and slightly lower on 'satiety responsiveness' [t(2365)=-3.625, p<0.001] than females, although the differences were small (d=0.149-0.218). Some small differences appeared to exist for 'enjoyment of food' for both measures of social class (NSSEC, F(2,2344)=8.488, p<0.001; maternal education, F(2,2352)=8.352, p<0.001) – in each case the infants from the 'high' social class categories were deemed to enjoy food less than those from either the 'low' (NSSEC, p=0.002; maternal education, p=0.008) or the 'middle' categories (NSSEC, p=0.009; maternal education, p=0.001), while the 'low' and 'middle' categories did not differ from one another in their enjoyment level (NSSEC, p=1.00; maternal education, p=1.00). In addition, infants from the 'high' category for NSSEC fed significantly more slowly than infants from the 'low' category (p=0.004), although the 'middle' category did not differ from the 'low' (p=0.906) or the 'high' groups (p=0.221). Nevertheless, all social class effects were very small (d=0.16-0.19).

No differences were found between MZ and DZ twins, different ethnic groups, or infants born to smokers or non-smokers for any of the appetitive traits; nor were scores associated with the age of the infants at BEBQ completion[45].

[45] Correlations between the BEBQ scales and age at BEBQ completion: 'enjoyment of food', ρ=-0.03, p=0.119, n=2355; 'food responsiveness', ρ=0.01, p = 0.779, n=2360; 'slowness in eating', ρ=-0.02, p=0.525, n=2372; 'satiety responsiveness', ρ=-0.04, p=0.057, n=2367; 'appetite size', ρ=-0.04, p=0.061, n=2373.

Table 6.9. Means (and standard deviations) for each scale of the BEBQ by sociodemographic and feeding characteristics

Variable of interest	Mean (sd)				
	EF	**FR**	**SE**	**SR**	**AS**
Zygosity					
MZ	4.29 (0.71)	1.92 (0.67)	2.78 (0.84)	2.47 (0.77)	3.21 (1.04)
DZ	4.27 (0.69)	1.96 (0.68)	2.70 (0.86)	2.42 (0.82)	3.31 (1.06)
Sex					
Males	4.29 (0.70)	**2.00** (0.70)	2.70 (0.85)	**2.38** (0.79)	**3.41** (1.08)
Females	4.27 (0.69)	**1.90** (0.65)	2.74 (0.86)	**2.50** (0.81)	**3.17** (1.03)
Gestational Age					
<34 weeks	**4.16** (0.79)	**1.77** (0.63)	**2.99** (0.97)	**2.63** (0.85)	**2.91** (1.08)
≥34 weeks	**4.29** (0.68)	**1.98** (0.68)	**2.68** (0.83)	**2.41** (0.80)	**3.34** (1.04)
Feeding Problems					
No	**4.38** (0.60)	**1.98** (0.68)	**2.61** (0.81)	**2.61** (0.84)	**3.38** (1.03)
Yes	**4.11** (0.80)	**1.91** (0.67)	**2.89** (0.89)	**2.33** (0.76)	**3.14** (1.08)
Feeding Method					
Breast-fed	4.35 (0.66)	**2.14** (0.70)	2.75 (0.82)	**2.27** (0.77)	**3.47**[a] (1.03)
Mixed-fed	4.26 (0.68)	**1.94**[a] (0.66)	2.67 (0.85)	**2.55**[a] (0.88)	**3.30**[ab] (1.03)
Bottle-fed	4.25 (0.71)	**1.85**[a] (0.65)	2.70 (0.87)	**2.50**[a] (0.80)	**3.20**[b] (1.06)
Smoking					
Yes	4.29 (0.65)	1.96 (0.72)	2.70 (0.88)	2.54 (0.87)	3.21 (1.09)
No	4.28 (0.70)	1.95 (0.67)	2.72 (0.85)	2.42 (0.80)	3.30 (1.05)
Ethnicity					
White British	4.27 (0.71)	1.94 (0.67)	2.73 (0.85)	2.43 (0.80)	3.28 (1.05)
Non-white British	4.29 (0.65)	2.03 (0. 71)	2.68 (0.86)	2.46 (0.84)	3.34 (1.09)
NSSEC					
Low	**4.36**[a] (0.65)	2.00 (0.75)	**2.62**[a] (0.88)	2.37 (0.80)	3.25 (1.15)
Middle	**4.35**[a] (0.65)	1.95 (0.72)	**2.68**[ab] (0.86)	2.41 (0.81)	3.40 (1.06)
High	**4.23** (0.72)	1.94 (0.645)	**2.76**[b] (0.85)	2.46 (0.81)	3.27 (1.03)
Maternal Education					
Low	**4.33**[a] (0.67)	1.93 (0.70)	2.63 (0.83)	2.41 (0.80)	3.21 (1.07)
Middle	**4.34**[a] (0.67)	1.90 (0.69)	2.75 (0.87)	2.44 (0.82)	3.26 (1.09)
High	**4.22** (0.72)	1.99 (0.66)	2.75 (0.86)	2.45 (0.80)	3.33 (1.04)

Abbreviations: EF, 'enjoyment of food'; FR, 'food responsiveness'; SE, 'slowness in eating'; SR, 'satiety responsiveness'; AS, 'appetite size'.

Sample sizes:
Zygosity: MZs, *n*=712-719; DZs, *n*=1578-1589.
Sex: males, *n*=1172-1183; females, *n*=1183-1192.
Gestational age: <34 weeks, *n*=311-315; >34 weeks, *n*=2033-2048.
Feeding problems: 'yes', *n*=919-928; 'no', *n*=1433-1445.
Feeding method: breast-fed, *n*=781-785; mixed-fed, *n*=221-224; bottle-fed, *n*=1257-1271.
Smoking during pregnancy: 'yes', *n*=262-265; 'no', *n*=2091-2107.
Ethnicity: White-British, *n*=1940-1959; non White-British, *n*=413-419.
National SSEC: 'low', *n*=451-455; 'middle', *n*=398-403; 'high', *n*=1497-1509.

Maternal education: 'low', *n*=502-507; 'middle', *n*=619-627; 'high', *n*=1234-1242.

Statistical information:
Groups whose means were significantly different are bolded.
[ab]Means sharing the same subscript are not significantly different from one another following Bonferroni correction for multiple comparisons (at an alpha level of $p<0.01$).

6.5. Discussion

6.5.1. Summary of findings

This chapter described the development of a new psychometric, parent-report measure of appetitive traits in the earliest period of life, during the milk-feeding phase. Four distinct dimensions of appetite emerged, with 17 items tapping four feeding traits, and one item describing overall 'appetite size'. 'Enjoyment of food' (4 items) relates to the infants' perceived liking of milk and of feeding in general, 'food responsiveness' (6 items) evaluates how demanding the infant is with regard to being fed and his or her level of responsiveness to cues of milk and feeding, 'slowness in eating' (4 items) measures the speed with which an infant typically feeds, and 'satiety responsiveness' (3 items) assesses how easily the infant gets full during a feed. The item 'My baby has a big appetite' correlated with all four of the scales, and may be used as a stand-alone item to measure overall 'appetite size'. The BEBQ components identified showed remarkable consistency with the CEBQ scales upon which they were based, and were the same constructs anticipated from the literature search and pilot work. The findings indicate that during the earliest period of life, before the infants have been introduced to solid foods, there are at least four distinguishable and measurable appetitive traits.

In keeping with studies of the CEBQ in children, the constructs that emerged were not entirely independent of one another and showed similar inter-correlation patterns as those observed in older samples. It appears to be the case that in both infants and children the appetitive scales cluster in a fairly coherent fashion. 'Satiety responsiveness' and 'slowness in eating' were positively correlated (0. 45), and the size of the correlation was only slightly smaller than that seen in older children (0.52 to 0.67) (Carnell & Wardle, 2007; Sleddens et al., 2008; Viana et al., 2008; Wardle et al., 2001b). The inter-correlation

between ‘satiety responsiveness and ‘slowness in eating’ across a number of age groups accords well with physiological theory about satiety – faster eating or feeding may outpace the natural satiety mechanisms which take several minutes to develop, and individuals who eat faster have sometimes also exhibited behaviours indicative of lower satiety sensitivity such as non-decelerated eating (e.g. Meyer & Pudel, 1972).

There was an inverse association between ‘food responsiveness’ and ‘satiety responsiveness’ (-0. 21), also smaller than at later ages (-0.36 to -0.51) (Sleddens et al., 2008; Viana et al., 2008), and a negative association between ‘food responsiveness’ and ‘slowness in eating’ (-0.10) which is also seen in older children (-0.23 to -0.53) (Viana et al., 2008; Wardle et al., 2001b). These relationships indicate that infants who are more demanding with regard to being fed and more responsive to cues of milk and feeding tend also to be less sensitive to internal satiety mechanisms and feed more quickly. ‘Enjoyment of food’ was negatively correlated with ‘satiety responsiveness’ (-0.45), although not as strongly as at later ages (-0.59 to -0.70) (Sleddens et al., 2008; Wardle et al., 2001b), suggesting that infants who like milk and feeding tend also to be lower in ‘satiety responsiveness’, perhaps as a result of reward pathways overriding basic appetite regulation, or as a result of faster feeding indicated by a moderate negative correlation between ‘enjoyment of food’ and ‘slowness in eating’ (-0.36) but which fell short of the extent of the clustering seen in older age groups (-0.47 to -0.64) (Carnell & Wardle, 2007; Sleddens et al., 2008; Viana et al., 2008; Wardle et al., 2001b).

‘Enjoyment of food’ and ‘food responsiveness’ were not significantly correlated (0.04), which was surprising given the size of the correlation observed between these two traits in children (0.44 to 0.78) (Carnell & Wardle, 2007; Sleddens et al., 2008; Viana et al., 2008; Wardle et al., 2001b), indicating that these characteristics may be independent of one another at this early age. The non-significant finding was not the result of lack of variation in ‘enjoyment of food’ as this trait showed moderate associations was the other eating behaviours (-0.36 for ‘slowness in eating, -0.45 for ‘satiety responsiveness’ and 0.34 for ‘appetite size’). It is possible that these two characteristics are independent of one another at this early age.

The single item measuring 'appetite size' showed moderate associations with all four of the scales such that children who enjoyed feeding more (0.34), were more food responsive (0.46), fed faster (-0.37) and were less sensitive to internal cues of satiety (-0.48), were also rated as having a bigger overall appetite, indicating that this item measures overall appetite avidity. Collectively, these findings indicate that the same dimensions of appetite that are seen in childhood are present at a very early age, and show similar interrelationships, although the dynamics of the associations are not as well-established as they are at older ages. This may be because the traits are biologically more distinct earlier in life, or because it is more difficult to distinguish between the different traits in a population who are only consuming milk as a result of less variation in behaviour.

It will be interesting to track eating behaviours from infancy into childhood to ascertain how stable they are over time and when the sizes of the correlations increase. There is strong tracking of the CEBQ appetitive traits between ages 4 and 11 (0.44 to 0.46) (Ashcroft et al., 2008), with children keeping their relative position at each age, although as a general rule there is a slight downward trend over time for 'satiety responsiveness' and 'slowness in eating' (with children becoming less satiety sensitive and increasing their eating speed as they get older), and a slight upward trend for 'enjoyment of food' and 'food responsiveness' (with children showing greater food responsiveness and increasing enjoyment of food over time) (Ashcroft et al., 2008). Moreover, a recent study reported that a single item measure of infant appetite at 6 weeks ('At present, how would you describe your baby's appetite'; 'very poor', 'poor', 'all right', 'good', 'very good') was positively associated with CEBQ 'enjoyment of food' and negatively with 'satiety responsiveness' measured 5-6 years later (Parkinson et al., 2010), although effect sizes were small suggesting that individual differences in appetite avidity that are manifested during the first few weeks may have some continuity but also some differences in the long-term. It is anticipated that the clustering of constructs will increase post- weaning as both dietary variety and choice increase.

It was noteworthy that the same component structure was reproduced in all of the subgroups examined despite a greatly reduced sample size in some cases (e.g. infants born before 34 weeks gestational age). This finding highlights the robustness of the BEBQ in that the same four distinct dimensions underlie infant appetite across infants with

different characteristics, including those with feeding problems. Nevertheless, the BEBQ was able to distinguish between some of the sub-groups in terms of mean scores. There were small sex differences with males being slightly more responsive to food (2.00 vs 1.90), slightly less sensitive to internal cues of satiety (2.38 vs 2.50), and having a larger appetite than female infants (3.41 vs 3.17), although the size of the difference was small in each case. Similarly, in a very large sample of 11-year old children Carnell and Wardle (2008a) found that males scored significantly lower on satiety sensitivity (and slowness in eating as a combined scale) measured using the CEBQ than females (2.60 vs 2.72), although they did not measure 'food responsiveness'. Smaller studies with children, with less statistical power to detect significant differences, have reported non-significant mean differences of a similar magnitude to those found in this study for 'food responsiveness' measured using the CEBQ (Sleddens et al., 2008; Wardle et al., 2001b). These findings suggest that there may be very small sex differences in some of these appetitive traits across development. There also appeared to be some very small social class effects. Infants from the higher social class category were scored as enjoying food slightly less than those from either the lower or middle categories; these same infants also fed more slowly than infants from the lower category. These findings raise the possibility that the social class effect that has sometimes been observed for infant weight may be mediated in part via appetitive mechanisms.

In this sample, greater prematurity was associated with lower appetite avidity, characterised by lower 'food responsiveness', lower 'enjoyment of food', a slower feeding pace, a higher sensitivity to satiety, and a smaller overall appetite. This finding supports evidence that smaller gestational age is associated with increasing slow growth during the first few months of life due to feeding problems and poor appetite which may be a consequence of immaturity and clinical instability (Cooke & Embleton, 2000; Cooke et al., 2004; Embleton et al., 2001). The same pattern of findings was observed for infants with reported feeding problems – they enjoyed food less, were less responsive to food, slower feeders, more satiety sensitive, and had a smaller overall appetite than infants without feeding problems.

There were some surprising findings related to feeding mode. Breast-fed infants tended to be more responsive to cues to feed and less satiety sensitive than either mixed-fed or

bottle-fed infants, and they had a larger overall appetite than those who were bottle-fed. This is unintuitive given the protective effect of breast-feeding on later obesity risk that has sometimes been observed in epidemiological studies (Gillman et al., 2001; Gluckman et al., 2007; Harder et al., 2005; Owen et al., 2005), and it goes against the hypothesis proposed by some researchers that one of the mechanisms through which breast-feeding attenuates weight gain is to upregulate the infant's satiety sensitivity by providing infants with better opportunity to control their intake, or because breast milk contains biologically active substances that promote satiety such as leptin (Bartok & Ventura, 2009). However, it may be the case that infants who had good appetites and fed well were more likely to be breast-fed than infants who appeared to have poorer appetites which accounts for the association.

Lastly, infants born slightly heavier were rated as enjoying food slightly more, feeding more quickly, being less satiety sensitive and having a larger overall appetite than infants born smaller. This is not surprising given the increased energy requirements of larger babies, although the effect was small in each case (0.11 to 0.16) showing also that the size infants are when they are born is not the most important driver of their appetite over the first three months of life.

6.5.2. Strengths and weaknesses

This study described the development of the first comprehensive measure of appetite in the earliest period of life, during the milk-feeding phase. The component structure and internal reliability of the scales were determined in a very large population-based sample of infants from over 2000 families across England and Wales. Nevertheless some limitations are acknowledged. The BEBQ is a parent-report measure and could be subject to bias. It would benefit from external validation, and a study is underway to examine associations with observed feeding behaviours. However, because it was based upon the CEBQ which has been validated using behavioural measures in children (Carnell & Wardle, 2007), and because the component structure and pattern of inter-correlations was the same, and because the component structure was reproduced across all subsamples of infants, the BEBQ can be used with some confidence. However, conclusions drawn from

PCA are necessarily restricted to the specific sample collected (Field, 2005), and replication of the component structure in singletons is needed to confirm the underlying dimensions of appetite. Likewise, the reliability of a scale can vary depending on the sample it is used with making it useful to repeat the analyses with other samples (Field, 2005), although the Cronbach's alphas were high for all scales across ten sub-groups indicating that this questionnaire is reliable. The BEBQ is currently being translated into a number of different languages which will make it possible to compare the internal validity and reliability of the questionnaire in different populations of infants.

6.5.3. Implications for theory, practice and future research

The findings from this study suggest that the underlying structure of appetite in infancy appears to be the same as that observed in older children. The Baby Eating Behaviour Questionnaire provides a comprehensive, convenient and easy-to-use measure of infant appetite during the milk-feeding phase that will allow large-scale research into predictors and outcomes of different appetitive traits. The traits captured in the BEBQ are based upon traits that have been associated with weight in older children and this instrument will be a useful tool for assessing the relationship with adiposity during this early stage of development. Study 2 in the next chapter explores associations with weight cross-sectionally. The BEBQ can also be used to explore the aetiology of infant appetite, in particular the relative influences of genes and the environment during this early period of development. Study 3 in Chapter 8 uses this new questionnaire to explore the heritability of these traits during the first three months of life.

CHAPTER 7. STUDY 2: ASSOCIATIONS BETWEEN APPETITE AND WEIGHT AT 3 MONTHS OF AGE

7.1. Background

There is large variability in child (and adult) weight despite the pressure of the current 'obesogenic' environment raising the possibility that some individuals are more susceptible to the temptations of the current environment than others (Carnell & Wardle, 2008a). At the same time individual differences in weight are largely explained by individual differences in genetic blueprints (Maes et al., 1997; Silventoinen et al., 2010). A behavioural susceptibility model of weight (as described in detail in Chapter 2) marries up what we know about weight heritability with variability in appetitive proclivities – inherited appetitive characteristics contribute to genetically-determined weight variability with certain individuals being more prone to overindulge in the abundance of food availability than others as a result of their genetic dispositions.

There has been some support for a behavioural susceptibility model of weight in the paediatric literature. In particular, differences in the appetitive traits captured in the CEBQ have been associated with differences in weight children – those children who demonstrate greater appetite avidity tend to have greater adiposity as well (Webber et al., 2009; Viana et al., 2008; Sleddens et al., 2008; Cunha et al., 2010; Carnell & Wardle, 2008a), and the same relationships have been found in older children (age 11) and younger children (age 4) (Carnell & Wardle, 2008a), highlighting that individual differences in appetitive traits are present from early on in the developmental trajectory. Not only have they been associated with weight but they have also been shown to have a heritable basis during childhood (Carnell et al., 2008).

There has been little research into how these characteristics relate to adiposity during infancy, hampered in part by the absence of a suitable psychometric measure to allow for convenient, large-scale measurement during this early period. However, appetite quality, measured at 6 weeks using one item, was found to predict weight gain between 0 and 12

months (Wright et al., 2006). In addition, two laboratory-based studies with infants associated sucking speed and sucking pressure for milk during the first few weeks of life with the development of adiposity over time (Agras et al., 1990; Stunkard et al., 2004). Furthermore, sucking speed (Stunkard et al., 2004) and sucking response to sweetened solution (Millstein, 1980) have been shown to distinguish infants at high risk of obesity from infants at low risk, given their parental weight status, implicating a causal role for these particular feeding behaviours in the development of weight, and suggesting that they are passed down from one generation to the next, potentially through genetic inheritance.

Although these studies provide convincing evidence that there are individual appetitive responses during early life that appear to relate to the development of weight over time, a drawback of the research is that the behaviours measured centred around sucking behaviour only. Also, given the observational nature of the designs, the samples were necessarily small, and only one or two feeds were analysed, so the underlying traits were inferred from the observed states during the laboratory sessions. A range of appetitive characteristics have been studied in relation to adiposity in children (not simply eating speed or bite size, akin perhaps to sucking speed or sucking pressure) and the development of the CEBQ (Wardle et al., 2001b) has allowed for large-scale research to relate these behaviours to weight (and to one another) by utilising parental opinion that is based upon aggregated observations of their child over multiple instances of the displayed behaviours. The development of the Baby Eating Behaviour Questionnaire described in the previous chapter has now provided the same opportunity to explore how a range of these traits relate to adiposity during the earliest period of life before any solid food has been introduced in a very large cohort of infants.

7.2. Study aims

The main objective of this study was to examine the relationships between appetitive traits measured during the first 3 months of life with weight at 3-months of life. In particular, I was interested in answering two questions:

1. Are appetitive traits during infancy associated with weight, and how do the magnitudes of the associations compare with those observed in children?

2. Are these traits independently associated with weight or do they appear to share a common pathway?

7.3. Methods

7.3.1. Measures

7.3.1.1. Appetite and weight

Eating styles during the first three months of infancy were characterised using mean scores from the four appetitive traits captured in the BEBQ (described in Chapter 6) including 'enjoyment of food', 'food responsiveness', 'slowness in eating', 'satiety responsiveness', plus the single item 'appetite size'. For ease of interpretation of the analyses in this chapter 'slowness in eating' and 'satiety responsiveness' were reversed so that higher scores for all BEBQ scales indicated greater appetite avidity, and positive associations between BEBQ scales and weight always indicate that a greater appetite predicts a higher weight. Because the BEBQ captured appetite during the first three months of life, adiposity at approximately 3 months of age was used to explore associations between the BEBQ scales and weight cross-sectionally. Adiposity was indexed using 3-month weight SDS. Weight change over the first three months of life was also of interest, and this was indexed by including birth weight SDS in the model thereby examining change in weight SD score from birth to three months (see Chapter 5, section 5.2.2.1.2. for a description of how birth weight SDS and 3-month weight SDS were calculated).

7.3.1.2. Potential covariates

A number of other potential predictors of 3-month weight were also measured so that they might be accounted for in the analyses as covariates should they be found to relate to both weight and appetite (associations between these variables and the BEBQ are reported in Chapter 6). Infant-related demographic variables included the age (in months and days) of

the infant when the BEBQ was completed, the age (in weeks) of the infant on the 3-month weight measurement occasion, gestational age (in weeks), sex, zygosity and ethnicity ('White-British' or 'non White-British'). Family social class was indexed using the National Statistics Socio-economic Classification (Office for National Statistics, 2000a; Office for National Statistics, 2000b) and maternal education, both collapsed into three classes ('low', 'intermediate' and 'high').

The effect of feeding problems ('yes' or 'no') and feeding method ('mainly breast-fed', 'mixed-fed' and 'mainly bottle-fed') were also of interest, as was maternal smoking during pregnancy ('yes' or 'no') (see Chapter 5, section 5.2.2.1 for a full description of how these variables were measured).

7.3.2. Statistical analyses

7.3.2.1. Identifying covariates

Associations with continuous variables and 3-month weight SD scores were evaluated using Pearsons' correlation coefficients (age at BEBQ completion, age at 3-month weight measurement occasion, and gestational age). Associations with categorical measures and 3-month weight SD scores were examined using independent samples t-tests for two-level variables (zygosity, sex, feeding problems, smoking during pregnancy, and ethnicity). Univariate analyses of variance (ANOVAs) were used to test for group differences across three-level variables (feeding method, maternal education and NS-SEC); significant group differences in the ANOVAs were followed up with pairwise posthoc comparisons using the Bonferroni correction for multiple tests to explore which groups were significantly different from one another[46]. Cohen's *r* was calculated for significant differences between two-level variables (including the post-hoc pairwise comparisons), and partial eta (analogous to Cohen's *r* (Tabachnick & Fidell, 2001)) for significant differences across three-level variables, to indicate the size of the effect across groups allowing for comparison with Pearson's correlations.

[46] An alpha-level of <0.01 was adopted to account for the large sample size.

7.3.2.2. Establishing the linearity of the relationship between the four appetitive traits, 'appetite size' and weight

In order to check that the appetitive traits share a linear relationship with weight in so far as appetite scores increase with each increase in weight in a linear fashion (to ensure the appropriateness of using a linear modeling technique), 3-month weight SD scores were grouped into quartiles with an equal number of infants in each group. Means and standard deviations of every appetitive trait were calculated for each weight quartile. A series of Complex Samples General Linear Models (CSGLMs) were run with each BEBQ scale as a continuous dependent variable and weight quartiles as a four level independent variable to test for differences in each BEBQ scale across the four quartiles of weight. CSGLMs can be used to build linear regression, multiple linear regression, analysis of variance, and analysis of covariance models, while taking into account the complex sample design (clustering of the twins within families), allowing the full sample of twins to be included in the analyses so that the power to detect small effects is maximised. A polynomial contrast test was included in the CSGLMs to examine whether any significant trends were identified across the groups for the appetitive traits, including linear, quadratic and cubic trends. Pairwise post hoc comparisons were carried out to explore which quartiles, if any, were significantly different from one another using the Bonferroni correction for multiple comparisons (an alpha-level of <0.01 was adopted to account for the large sample size). The CSGLMs were rerun including the covariates (described above) to check that the results were the same.

7.3.2.3. Establishing relationships between the appetitive traits and weight as a continuous measure

For the following analyses all of the appetite scores and 3-month weight SD scores were standardised into z-scores so that they all had a mean of 0 and a standard deviation of 1. This meant that the predictive value of each appetite scale for 3-month weight SD score could be better compared.

Associations between the four BEBQ scales and 'appetite size' and 3-month weight SDS as a continuous measure were examined first using simple pairwise Pearson's product moment correlation coefficients. A series of CSGLMs were then run with 3-month weight SD z-score as the continuous dependent variable in each case, and one BEBQ trait z-score included at a time as a continuous independent variable, adjusting for the covariates (described above)[47]. For each analysis the total adjusted R^2 is presented which indicates the total amount of variance in 3-month weight SDS explained by all of the variables included in the model. In addition, parameter estimates (the beta coefficient of each predictor variable), standard errors for the estimates, 95% confidence intervals for the estimates, and t-tests[48] for each estimate are reported for all of the BEBQ traits. Because the BEBQ scales have all been standardized the beta values may be compared across models to identify if certain BEBQ traits have a greater predictive value for 3-month weight than others – a parameter estimate that sits outside the 95% confidence interval of the other estimates was considered significantly different. The t-value is another indication of the relative effect size of each appetitive trait on 3-month weight SD score.

The simple bivariate association between birth weight SDS and 3-month weight SDS as a continuous measure was examined using Pearson's product moment correlation coefficient. The CSGLMs were then rerun also including birth weight SDS in the model to investigate if the BEBQ scales and 'appetite size' were related to change in weight SD score from birth to three months.

7.3.2.4. Establishing the independence of the relationships between the appetitive traits and weight

Lastly, in order to evaluate whether the appetitive characteristics influence weight independently of one another, or through a common pathway, a CSGLM was run with 3-month weight SD z-score as the dependent variable, and the five appetitive traits entered

[47] The CSGLMs were also run including each potential two-way interaction (one at a time) between the BEBQ traits and all of the covariates. Only one two-way interaction was found - lower satiety sensitivity was associated with slightly lower weight for breast-fed babies but higher weight for mixed-fed and bottle-fed babies, but the effect size was very small. Because only one two-way interaction effect was found to be significant out of 30 effects that were tested (5 scales, 6 interaction effects for each scale), and the effect size was very small, the results from the models including only the main effects are reported.

[48] The null hypothesis for each t-test is that the value of the coefficient is 0.

simultaneously. In order to obtain as much information as possible a hierarchical model was set up: the covariates were entered first, the four distinct appetitive traits were entered second ('enjoyment of food', 'food responsiveness', 'slowness in eating' and 'satiety responsiveness'), and lastly 'appetite size' was entered into the model to see the extent to which the four appetitive traits influence weight independently of overall appetite size. These analyses were repeated including birth weight in each step of the model to ascertain which, if any, of the appetitive traits shared an independent relationship with change in weight SD score from birth to 3 months. The same statistics outlined in section 7.3.2.3. were reported for this analysis.

7.4. Results

3-month weight data were available for 4214 infants. As expected for twins, the mean weight SD score for the sample was less than 0 at -0.28 (range, -5.49 to 4.26) indicating that the infants had lower weights compared to general UK population norms.

7.4.1. Identification of covariates

A number of factors were associated with 3-month weight SD scores (Tables 7.1 and 7.2). Although 3-month weight SD scores are already adjusted for gestational age, this factor was nevertheless significantly and positively associated with weight such that infants born later were slightly heavier, although the size of the effect was very small. The same was true of sex – boys were slightly heavier than girls, and the difference was significant [$t(4212)=3.680$, $p<0.001$] but the effect size was only small.

Table 7.1. Pearson's correlation coefficients showing associations between potential covariates and 3-month weight SD scores

BEBQ Scale	*r*[1]	*p*-value	*n*
Age at BEBQ completion	-0.03	0.035	4214
Age at 3-month weight measurement occasion	-0.01	0.524	4214
Gestational age	**0.05**	**0.001**	4214

[1]Small effect size, *r*=0.1–0.23; medium, *r*=0.24–0.36; large, *r*=0.37 or larger (Cohen 1988, 1992).

Unsurprisingly, infants with reported feeding problems were significantly smaller at 3-months than those with no reported feeding problems [$t(3272.486)=7.057$, $p<0.001$], but the effect size was not large. In this sample, there was a significant effect of feeding method on weight at 3 months [$F(2,4035)=80.127$, $p<0.001$]; infants who had been mixed-fed or bottle-fed during the first three months of life were significantly heavier at 3 months than infants who had been breast-fed during the first three months ($p<0.001$ in each case), and the sizes of the effects were small (r=-0.20 to -0.21).

Weight differed across maternal education [$F(2,4211)=8.008$, $p<0.001$]. In particular, infants born to mothers with 'low' education were significantly heavier than infants born to mothers with 'high' education ($p<0.001$), but the size of the effect was very small (r=0.08); the mean weight of the 'intermediate' group was not significantly different from that of the 'low' (p=0.181) or the 'high' group (p=0.156), although it was in between. Weight also differed with the other index of social class (NS-SEC) [$F(2,4197)=5.800$, p=0.003]. Infants born into households that were classified as 'low' or 'intermediate' were slightly heavier than infants born into households considered 'high', although these differences were not considered significant (p=0.023 and p=0.025).

The variables found here to predict 3-month weight SD scores (gestational age, feeding method, maternal education, NS-SEC, sex and feeding problems) were also found to be significantly associated with appetite scores in Chapter 6 (although the direction of the relationship between feeding method and weight found here was different to that between feeding method and appetite reported in Chapter 6), so these variables were included in the CSGLMs as covariates.

Table 7.2. Means (standard deviations) and significant differences between groups for potential covariates

Characteristic	mean[1] (sd)	*n*	*p*-value	effect size[2]
Zygosity				
MZ	-0.28 (1.10)	1270	0.976	
DZ	-0.28 (1.08)	2821		
Sex				
Male	**-0.22** (1.11)	2071	**<0.001**	**0.06**
Female	**-0.34** (1.07)	2143		
Feeding Problems				
No	**-0.18** (1.04)	2561	**<0.001**	**0.11**
Yes	**-0.43** (1.15)	1651		
Feeding Method				
Breast-fed	**-0.56** (1.11)	1410	**<0.001**	**0.19**
Mixed-fed	**-0.09**[a] (1.08)	396		
Bottle-fed	**-0.12**[a] (1.04)	2232		
Smoking				
No	-0.27 (1.09)	3775	0.148	
Yes	-0.35 (1.08)	437		
Maternal Education				
Low	**-0.16**[a] (1.08)	860	**<0.001**	**0.06**
Intermediate	-0.26[ab] (1.12)	1097		
High	**-0.34**[b] (1.08)	2257		
NS-SEC				
Low	**-0.20** (1.09)	769	**0.003**	**0.05**
Intermediate	**-0.20** (1.08)	696		
High	**-0.32** (1.09)	2735		
Ethnicity				
White British	-0.27 (1.08)	3499	0.069	
Non White British	-0.35 (1.11)	715		

[1] Groups whose means were significant different are bolded.

[2] Effect size reported for t-test analyses is *r*, which is equivalent to Pearson's correlation coefficient (small effect size, *r*=0.1–0.23; medium, *r*=0.24–0.36; large, *r*=0.37 or larger (Cohen 1988, 1992)). *r* was calculated from the t-value and the df using the following formula: $r = \sqrt{(t^2/ t^2+df)}$ (Field, 2005). Effect size reported for ANOVAs is partial eta for between subjects effect, which is analogous to *r* (Tabachnick & Fidell, 2001).

[ab] Means sharing the same subscript are not significantly different from one another following Bonferroni correction for multiple comparisons (at an alpha level of $p<0.01$).

7.4.2. Linearity of appetite scores across 3-month weight SD quartiles

The first quartile included infants with the lowest weight SD scores and the fourth quartile contained infants with the highest weight SD scores: first quartile = <-0.9715 (*n*=1053; mean=-1.67); second quartile = ≥-0.9715 and <-0.2513 (*n*=1054; mean=-0.58); third quartile = ≥-0.2513 and <0.4280 (*n*=1054; mean=0.07); fourth quartile = ≥0.4280 (*n*=1053; mean=1.06). The mean scores (and standard deviations) for each BEBQ scale and 'appetite size' by 3-month weight SDS quartile are shown in Table 7.3. As can be seen, for each appetite scale the lowest means are observed in the first quartile of weight, and the scores increase in a graded fashion across each quartile with the highest means being demonstrated by the highest quartile of weight. The univariate CSGLMs showed that weight quartile significantly predicted all of the appetitive traits: 'enjoyment of food , $F(3, 2086)=18.180$, $p<0.001$; 'food responsiveness', $F(3, 2091)=9.300$, $p<0.001$; 'slowness in eating', $F(3, 2099)=42.570$, $p< 0.001$; 'satiety responsiveness', $F(3, 2097)=34.582$, $p< 0.001$; and 'appetite size', $F(3, 2099)=80.316$, $p<0.001$.

Polynomial contrasts showed that there were significant linear trends across the 3-month weight quartiles for all of the scales (Table 7.3): 'enjoyment of food', $F(1, 2088)=53.893$, $p<0.001$; 'food responsiveness', $F(1, 2093)=23.795$, $p<0.001$; 'slowness in eating', $F(1, 2101)=127.074$, $p<0.001$; 'satiety responsiveness', $F(1, 2099)=99.622$, $p<0.001$; 'appetite size', $F(1, 2101)=232.879$, $p<0.001$. These findings indicated that as weight increased, appetitive traits increased proportionately. No significant quadratic[49] or cubic trends[50] were found across the groups for any of the traits.

For 'slowness in eating', 'satiety responsiveness' and 'appetite size' post hoc pairwise comparisons revealed that all four groups were significantly different from one another ($p<0.001$ to $p=0.007$). The same tests indicated that for 'enjoyment of food' most of the groups were significantly different from one another except for the first quartile with the

[49] Quadratic trend statistics: 'enjoyment of food', $F(1,2088)=0.001$, $p=0.977$; 'food responsiveness', $F(1,2093)=4.631$, $p=0.032$; 'slowness in eating', $F(1,2101)=0.392$, $p=0.531$; 'satiety responsiveness', $F(1,2099)=0.556$, $p=0.456$; 'appetite size', $F(1,2101)=2.867$, $p=0.091$.
[50] Cubic trend statistics: 'enjoyment of food', $F(1,2088)=0.288$, $p=0.591$; 'food responsiveness', $F(1,2093)=2.017$, $p=0.156$; 'slowness in eating', $F(1,2101)=0.033$, $p=0.855$; 'satiety responsiveness', $F(1,2099)=0.446$, $p=0.504$; 'appetite size', $F(1,2101)=3.810$, $p=0.051$.

second, and the second quartile with the third, and for ‘food responsiveness’ all groups were significantly different from the fourth quartile ($p<0.001$). The results were the same after adjustment for the covariates.

Table 7.3. Means (standard deviations) and linear trends of appetitive traits across 3-month weight SDS quartiles

BEBQ Scale	Mean (sd) *n*				*p*-value (linear contrast effect)	
	First quartile	Second quartile	Third quartile	Fourth quartile	Model 1	Model 2
'Enjoyment of food'	**4.14[a]**	**4.24[ab]**	**4.30[b]**	**4.40**	**<0.001**	**<0.001**
	(0.79)	(0.74)	(0.62)	(0.63)	(0.184)	(0.177)
	1043	1035	1044	1043		
'Food Responsiveness'	**1.88[a]**	**1.91[a]**	**1.93[a]**	**2.06**	**<0.001**	**<0.001**
	(0.64)	(0.67)	(0.64)	(0.73)	(0.121)	(0.156)
	1046	1046	1046	1037		
'Slowness in Eating'	**3.04**	**3.20**	**3.36**	**3.50**	**<0.001**	**<0.001**
	(0.86)	(0.83)	(0.84)	(0.84)	(0.344)	(0.322)
	1049	1049	1046	1046		
'Satiety Responsiveness'	**3.36**	**3.49**	**3.59**	**3.76**	**<0.001**	**<0.001**
	(0.88)	(0.80)	(0.77)	(0.74)	(0.292)	(0.317)
	1046	1050	1047	1044		
'Appetite Size'	**2.92**	**3.16**	**3.33**	**3.69**	**<0.001**	**<0.001**
	(1.08)	(1.02)	(0.99)	(0.98)	(0.558)	(0.587)
	1049	1050	1043	1049		

[ab] Pairwise means sharing the same superscript are not significantly different from one another in the univariate models, after adjustment for multiple comparisons (p<0.01, Bonferroni).

Model 1: unadjusted model which includes only 3-month weight SDS quartiles as predictor and appetitive trait as the dependent variable.

Model 2: appetitive trait for each 3-month weight SDS quartile, adjusted for gestational age, feeding method, NSSEC, maternal education, sex and feeding problems.

7.4.3. Associations between appetitive traits and 3-month weight SD as a continuous outcome

Table 7.4 shows simple bivariate associations between each appetitive trait and 3-month weight SD score. All appetitive traits were significantly related to weight SD score at 3 months. The effect sizes appeared to differ slightly – correlations with 'enjoyment of food' and 'food responsiveness' were somewhat smaller than those with 'slowness in eating', 'satiety responsiveness' and 'appetite size'. All correlations were considered 'small', except for the association between 'appetite size' and 3-month weight SD score which was medium in magnitude, according to Cohen's guidelines (Cohen, 1988; Cohen, 1992).

Table 7.4. Pearson's correlation coefficients showing associations between appetitive traits and 3-month weight SD scores

BEBQ Scale	*r*	*p*-value	*n*
'Enjoyment of Food'	0.14	<0.001	4165
'Food Responsiveness'	0.10	<0.001	4175
'Slowness in Eating'	0.20	<0.001	4190
'Satiety Responsiveness'	0.19	<0.001	4187
'Appetite size'	0.29	<0.001	4191

Small effect size, *r*=0.1–0.23; medium, *r*=0.24–0.36; large, *r*=0.37 or larger (Cohen, 1988; Cohen, 1992).

The CSGLMs indicated that after adjustment for the covariates, the associations between each of the appetitive traits (entered into separate models) and 3-month weight SD scores were still significant (Table 7.5). The ranking of the beta values was the same as the ranking of the *r*-values from the simple correlations; 'slowness in eating', 'satiety responsiveness' and 'appetite size' all had beta values that were higher than (and outside the 95% confidence intervals of) the beta estimates for 'enjoyment of food' and 'food responsiveness'. Moreover, 'appetite size' had the greatest predictive value of all the traits for 3-month weight SDS as the beta was substantially higher than the others and the 95% confidence interval did not overlap with any other intervals. The R^2 and t-values are other indicators of the relative effects sizes and both statistics support this conclusion – the

greatest proportion of variance in 3-month weight SD is explained by a model with the covariates and 'appetite size' (which explained 14% of the variance in 3-month weight SDS, versus 7-9% from models including the other appetite scales), and the t-value for the beta estimate for 'appetite size' was the largest. In no cases was there a clustering effect of the twins in families as this parameter was estimated as 0 for each model.

Table 7.5. Associations between appetitive traits and 3-month weight SDS, following adjustment for covariates

BEBQ Scale	Beta (SE)	Beta 95% CI	R^2	t	*p*-value	*n*
'Enjoyment of food'	0.14 (0.02)	0.10,0.17	0.07	7.398	<.001	3979
'Food Responsiveness'	0.14 (0.02)	0.10,0.18	0.07	7.187	<.001	3990
'Slowness in Eating'	0.19 (0.02)	0.15,0.23	0.08	10.109	<.001	4005
'Satiety Responsiveness'	0.21 (0.02)	0.17,0.24	0.09	11.431	<.001	3999
'Appetite Size'	0.31 (0.02)	0.28,0.35	0.14	16.993	<.001	4003

Covariates include gestational age, feeding method, maternal education, NS-SEC, sex and feeding problems.

Birth weight SD score was significantly associated with 3-month weight SD score (r=0.56, p<0.001, n=4177) in the simple bivariate correlation, and the effect size was fairly large. After birth weight was added in to the CSGLMs along with the other covariates it was a significant predictor of 3-month weight SD score, but the associations between the five appetite traits and 3-month weight SD score remained significant as well showing that each of these appetitive characteristics significantly predicted change in weight SD score from birth to 3 months[51]. As would be expected, the sizes of the betas in each case were attenuated in comparison to the model without birth weight, although as before the betas were smallest for 'enjoyment of food' [Beta=0.08 (SE=0.02; 95% confidence interval=0.05, 0.11), R^2=0.39, t(1987)=5.143, p<0.001] and 'food responsiveness' [Beta=0.09 (SE=0.02; 95% CI=0.06, 0.13), R^2=0.39, t(1992)=5.515, p<0.001], and largest for 'appetite size' [Beta=0.21 (SE=0.02, 95% CI=0.18, 0.24), R^2=0.42, t(1999)=12.978, p<0.001]; betas

[51] 'Enjoyment of food', F(1,1987)=26.449, p<0.001; 'food responsiveness', F(1,1992)=30.418, p<0.001; 'slowness in eating', F(1,2000)=55.013, p<0.001; 'satiety responsiveness', F(1,1997)=56.269, p<0.001; 'appetite size', F(1,1999)=168.419, p<0.001.

were in between for 'slowness in eating' [Beta=0.12 (SE=0.02; 95% CI=0.09, 0.15), R^2=0.39, t(2000)=7.417, p<0.001] and 'satiety responsiveness' [Beta=0.12 (SE=0.02; 95% CI=0.09, 0.15), R^2=0.39, t(1997)=7.501, p<0.001]. There was no clustering effect of the twins in families for any of the models as this parameter was estimated as 0 in each case.

7.4.4. Independent pathways between appetitive traits and 3-month weight SD scores

The results of the hierarchical CSGLMs are shown in Tables 7.6 to 7.8. The covariates (but not birth weight) were entered into the model first; when these variables were entered simultaneously only feeding method, sex and feeding problems remained significant predictors of weight at 3 months (Model 1, Table 7.6)[52] – infants without feeding problems were heavier than those with feeding problems, males weighed more than females, and breast-fed infants were significantly lighter than bottle-fed infants (mixed-fed infants did not differ significantly in weight from bottle-fed infants). Together, these factors accounted for just over 5% of the variance in 3-month weight SD scores. There was no clustering effect of the twins in families (this parameter was estimated as 0).

When birth weight was added into the CSGLM with the other covariates the results were very similar, although gestational age also became significant such that infants born later had a slightly greater change in weight SD score from birth to three months[53]. In keeping with the previous model males had a greater change in weight than females, as did infants without feeding problems, and breast-fed infants had a smaller change in weight SD score than bottle-fed infants (mixed-fed infants did not differ significantly in weight change from bottle-fed infants). There was no clustering effect of the twins in families for weight change (this parameter was estimated as 0). This model explained 38% of the variance in 3-month weight SDS (R^2=0.38).

[52] Gestational age, F(1,2021)=0.003, p=0.953; feeding method, F(2,2020)=48.303, p<0.001; NS-SEC, F(2,2020)=0.280, p=0.756; maternal education, F(2,2020)=0.607, p=0.545; sex, F(1,2021)=14.253, p<0.001; feeding problems, F(1,2021)=27.429, p<0.001.
[53] Birth weight, F(1,2008)=1418.515, p<0.001; gestational age, F(1,2008)=60.743, p <0.001; feeding method, F(2,2007)=59.106, p<0.001; NS-SEC, F(2,2007)=0.387, p=0.679; maternal education, F(2,2007)=0.885, p=0.413; sex, F(1,2008)=15.621, p<0.001; feeding problems, F(1,2008)=11.066, p=0.001.

Table 7.6. Independent associations of covariates and 3-month weight SDS

Predictor Variables		Model 1[1] (*n*=4024)			
		Beta (SE)	Beta 95% CI	t	*p*-value
Gestational age		0.00 (0.01)	-0.02,0.02	-0.059	0.953
Feeding Method[2]	**–** breast-fed	**-0.39 (0.04)**	**-0.48,-0.31**	**-9.241**	**<0.001**
	– mixed-fed	0.06 (0.07)	-0.07,0.19	0.854	0.393
NSSEC3[3]	**–** low	0.02 (0.06)	-0.09,0.12	0.304	0.761
	– intermediate	0.04 (0.06)	-0.07,0.15	0.742	0.458
Maternal education[3]	**–** low	0.05 (0.05)	-0.06,0.16	0.939	0.348
	– intermediate	-0.01 (0.05)	-0.11,0.09	-0.182	0.856
Sex – Male		**0.13 (0.03)**	**0.06,0.19**	**3.775**	**<0.001**
No feeding problems		**0.21 (0.04)**	**0.13,0.29**	**5.237**	**<0.001**

[1] R^2 = 0.05.
[2] Reference group is 'bottle-fed'.
[3] Reference group is 'high'.

When the four distinct BEBQ scales were added into the model following the covariates (but not birth weight), only 'food responsiveness', 'slowness in eating' and 'satiety responsiveness' remained significant predictors of 3-month weight SD scores, but 'enjoyment of food' was no longer significant in the presence of the others (Model 2, Table 7.7)[54]. This indicated that most of the covariance shared between 'enjoyment of food' and weight is also shared with the other appetitive traits, whereas most of the covariance between each of the other three characteristics and weight is independent. Adding these four eating behaviours into the model explained an additional 7% of the variance in weight SD scores on top of the covariates (with a total of 12% of the variance being explained by all of the factors in the model). Still, there was no clustering effect of the twins in families as this parameter was estimated as 0. Feeding method and feeding problems remained significant predictors of 3-month weight but the other covariates did not significantly predict it[55].

[54] 'Enjoyment of food', $F(1,1985)=1.356$, $p=0.244$; 'food responsiveness', $F(1,1985)=31.890$, $p<0.001$; 'slowness in eating', $F(1,1985)=28.645$, $p<0.001$; 'satiety responsiveness', $F(1,1985)=32.074$, $p<0.001$.
[55] Gestational age, $F(1,1985)=2.144$, $p=0.143$; feeding method, $F(2,1984)=69.145$, $p<0.001$; NS-SEC, $F(2,1984)=0.323$, $p=0.724$; maternal education, $F(2,1984)=0.099$, $p=0.905$; sex, $F(1,1985)=5.533$, $p=0.019$; feeding problems, $F(1,1985)=10.333$, $p=0.001$.

The results were very similar after birth weight was added in to the CSGLM along with the other covariates and the four BEBQ scales – 'enjoyment of food' did not significantly predict change in weight SD score from birth to 3 months in the presence of 'food responsiveness', 'slowness in eating' and 'satiety responsiveness' which were all significant predictors of weight change[56]. Again, the sizes of the betas were smaller for most scales than those for the model without birth weight ['enjoyment of food': Beta=0.02 (SE=0.02; 95% CI=-0.02, 0.05), t(1972)=1.082, p=0.279; 'food responsiveness': Beta=0.08 (SE=0.02; 95% CI=0.05, 0.11), t(1972)=4.698, p< 0.001; 'slowness in eating': Beta=0.08 (SE=0.02; 95% CI=0.04, 0.11), t(1972)=4.134, p <0.001], 'satiety responsiveness' [Beta=0.06 (SE=0.02; 95% CI=0.02, 0.10), t(1972)=3.299, p<0.001]. Being male, being bottle-fed or mixed-fed, and being born later all significantly predicted greater weight change, independently of the four BEBQ scales[57], but none of the other covariates were significant. As before, there was no clustering effect of the twins in families. The model explained 40% of the variance in 3-month weight SD score (R^2=0.40), suggesting that the four appetitive traits explained 2% of the variance in weight change from birth to 3 months.

[56] 'Birth weight', $F(1,1972)=1199.941$, $p<0.001$; 'Enjoyment of food', $F(1,1972)=1.171$, $p=0.279$; 'food responsiveness', $F(1,1972)=22.073$, $p<0.001$; 'slowness in eating', $F(1,1972)=17.091$, $p<0.001$; 'satiety responsiveness', $F(1,1972)=10.883$, $p=0.001$.

[57] Gestational age, $F(1,1972)=36.619$, $p<0.001$; feeding method, $F(2,1971)=73.301$, $p<0.001$; NS-SEC, $F(2,1971)=0.015$, $p=0.985$; maternal education, $F(2,1971)=0.478$, $p=0.620$; sex, $F(1,1972)=9.594$, $p=0.002$; feeding problems, $F(1,1972)=4.593$, $p=0.032$.

Table 7.7. Independent associations of 'enjoyment of food', 'food responsiveness', 'slowness in eating' and 'satiety responsiveness' entered simultaneously, and 3-month weight SDS, following adjustment for covariates

Predictor Variables		Model 2[1] (*n*=3945)			
		Beta (SE)	Beta 95% CI	t	*p*-value
Gestational age		-0.01 (0.01)	-0.03,0.00	-1.464	0.143
Feeding Method[2]	– breast-fed	**-0.49 (0.04)**	**-0.56,-0.40**	**-11.232**	**<0.001**
	– mixed-fed	0.03 (0.06)	-0.09,0.16	0.532	0.595
NSSEC3[3]	– low	0.04 (0.05)	-0.15,0.06	-0.786	0.432
	– intermediate	-0.00 (0.06)	-0.11,0.11	-0.054	0.957
Maternal education[3]	– low	0.02 (0.05)	-0.08,0.13	0.403	0.687
	– intermediate	-0.00 (0.05)	-0.10,0.10	-0.026	0.979
Sex – Male		0.08 (0.03)	0.01,0.14	2.352	0.019
Feeding Problems		**0.13 (0.04)**	**0.05,0.21**	**3.21**	**0.001**
'Enjoyment of food'		0.02 (0.02)	-0.02,0.07	1.165	0.244
'Food Responsiveness'		**0.11 (0.02)**	**0.07,0.15**	**5.647**	**<0.001**
'Slowness in Eating'		**0.12 (0.02)**	**0.07,0.16**	**5.352**	**<0.001**
'Satiety Responsiveness'		**0.13 (0.02)**	**0.08,0.17**	**5.663**	**<0.001**

[1] Model 2: $R^2 = 0.12$.
[2] Reference group is 'bottle-fed'.
[3] Reference group is 'high'.

Adding 'appetite size' into the model with the covariates (but not birth weight) and the four other BEBQ scales resulted in neither 'enjoyment of food' nor 'food responsiveness' significantly predicting 3-month weight, while the predictive values of 'slowness in eating' and 'satiety responsiveness' remained significant but greatly attenuated as indicated by the smaller beta values and t-values compared to Model 2 (Model 3, Table 7.8)[58]. These findings suggest that the covariance between 'food responsiveness' and 3-month weight is entirely explained by the covariance shared between 'appetite size' and 3-month weight; on the other hand, only part of the effects of 'slowness in eating' and 'satiety responsiveness' on 3-month weight are shared with the relationship between 'appetite size' and weight, and some of their effects on 3-month weight are independent of 'appetite

[58] 'Enjoyment of food', $F(1,1981)=0.736$, $p=0.391$; 'food responsiveness', $F(1,1981)=0.619$, $p=0.432$; 'slowness in eating', $F(1,1981)=12.035$, $p=0.001$; 'satiety responsiveness', $F(1,1981)=9.499$, $p=0.002$; 'appetite size', $F(1,1981)=109.647$, $p<0.001$.

size'. Unsurprisingly, adding 'appetite size' into the model yielded a model that explained a greater proportion of variance in 3-month weight SD scores with this variable explaining another 3% on top of Model 2; collectively the five appetitive traits explained 10% of the variance in 3-month weight SD scores, after adjustment for the covariates. Again, there was no clustering effect of the twins in families as this parameter was estimated as 0. Feeding method and feeding problems remained significant predictors of 3-month weight but the other covariates did not significantly predict it[59].

Lastly, when birth weight was added into the model along with the covariates and all of the appetite scores only 'appetite size' significantly predicted change in weight SD score from birth to 3 months[60] ['enjoyment of food': Beta=-0.01 (SE=0.02; 95% CI=-0.05, 0.02), $t(1968)=-0.660$, $p=0.509$; 'food responsiveness': Beta=0.01 (SE=0.02; 95% CI=-0.02, 0.05), $t(1968)=0.721$, $p=0.471$; 'slowness in eating': Beta=0.05 (SE=0.02; 95% CI=0.01, 0.08), $t(1968)=2.559$, $p=0.011$; 'satiety responsiveness' (Beta=0.02 (SE=0.02; 95% CI=-0.02, 0.06), $t(1968)=1.119$, $p=0.263$; 'appetite size' (Beta=0.18 (SE=0.02; 95% CI=0.14, 0.22), $t(1968)=9.242$, $p<0.001$]. These findings suggest that the covariance between all of the other BEBQ scales and change in weight SD score from 0 to 3 months are explained by the covariance shared between 'appetite size' and change in weight SD score from 0 to 3 months. Being born later and being bottle-fed or mixed-fed predicted significantly greater change in weight SD score from birth to 3 months independently of the five appetitive traits, but the other covariates were not significant predictors of weight change[61]. Again, there was no clustering effect of the twins in families as this parameter was estimated as 0. A model that included the covariates, birth weight and the five appetitive traits together explained the greatest proportion of variance ($R^2=0.42$); together, the five appetitive traits explained 4% of the variance in weight change from birth to 3 months, after adjustment for the covariates.

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[59] Gestational age, $F(1,1981)=4.096$, $p=0.043$; feeding method, $F(2,1980)=70.719$, $p<0.001$; NS-SEC, $F(2,1980)=0.281$, $p=0.755$; maternal education, $F(2,1980)=0.459$, $p=0.632$; sex, $F(1,1981)=2.080$, $p=0.149$; feeding problems, $F(1,1981)=11.596$, $p=0.001$.

[60] 'Enjoyment of food', $F(1,1968)=0.436$, $p=0.509$; 'food responsiveness', $F(1,1968)=0.520$, $p=0.471$; 'slowness in eating', $F(1,1968)=6.550$, $p=0.011$; 'satiety responsiveness', $F(1,1968)=1.252$, $p=0.263$; 'appetite size', $F(1,1968)=85.412$, $p<0.001$.

[61] Gestational age, $F(1,1968)=33.109$, $p< 0.001$; feeding method, $F(2,1967)=75.373$, $p<0.001$; NS-SEC, $F(2,1967)=0.180$, $p=0.835$; maternal education, $F(2,1967)=0.915$, $p=0.401$; sex, $F(1,1968)=5.445$, $p=0.020$; feeding problems, $F(1,1968)=5.367$, $p=0.021$.

Table 7.8. Independent associations of all of the appetitive traits entered simultaneously and 3-month weight SDS, following adjustment for covariates

Predictor Variables		**Model 3[1] (*n*=3937)**			
		Beta (SE)	Beta 95% CI	t	*p*-value
Gestational age		-0.02 (0.01)	-0.04,0.00	-2.024	0.043
Feeding Method[2]	**–** breast-fed	**-0.49 (0.04)**	**-0.57,-0.41**	**-11.422**	**<0.001**
	– mixed-fed	0.02 (0.06)	-0.10,0.14	0.324	0.746
NSSEC3[3]	**–** low	-0.04 (0.05)	-0.14,0.07	-0.648	0.517
	– intermediate	-0.03 (0.06)	-0.14,0.08	-0.542	0.588
Maternal education[3]	**–** low	0.05 (0.05)	-0.06,0.15	0.913	0.361
	– intermediate	0.00 (0.05)	-0.09,0.10	0.074	0.941
Sex – Male		0.05 (0.03)	-0.02,0.11	1.442	0.149
Feeding Problems		**0.14 (0.04)**	**0.06,0.22**	**3.405**	**0.001**
'Enjoyment of food'		-0.02 (0.02)	-0.06,0.02	-0.858	0.391
'Food Responsiveness'		0.02 (0.02)	-0.03,0.06	0.787	0.432
'Slowness in Eating'		**0.08 (0.02)**	**0.03,0.12**	**3.469**	**0.001**
'Satiety Responsiveness'		**0.07 (0.02)**	**0.03,0.12**	**3.082**	**0.002**
'Appetite Size'		**0.25 (0.02)**	**0.21,0.30**	**10.471**	**<0.001**

[1] Model 3: $R^2 = 0.15$.
[2] Reference group is 'bottle-fed'.
[3] Reference group is 'high'.

7.5. Discussion

7.5.1. Summary of findings

This study set out to examine the relationship between a range of appetitive traits measured during the first three months of life, and weight at 3 months of age. In particular, the analyses in this chapter served to answer two questions, discussed below:

1. Are appetitive traits during infancy associated with weight, and how do the magnitudes of the associations compare with those observed in children?
2. Are these traits independently associated with weight or do they appear to share a common pathway?

7.5.1.1 Are appetitive traits during infancy associated with weight, and how do the magnitudes of the associations compare with those observed in children?

All of the appetitive characteristics captured in the BEBQ were associated with weight at 3-months in the simple bivariate analyses – greater enjoyment of feeding and milk (r=0.14), greater food responsiveness (r=0.10), faster feeding (r=0.20), lower sensitivity to internal cues of satiety (r=0.19) and a larger overall appetite (r=0.29) were characteristics of infants with higher weight at 3 months of age. The relationships were unchanged by adjustment for covariates and clustering of the twins in families, and the significant associations remained (although attenuated) after adjustment for birth weight, indicating that appetitive characteristics during the first three months of life also predict the rate at which an infant grows during this period of time. Collectively, the four BEBQ traits and 'appetite size' explained 10% of the variance in 3-month weight SD score, and 4% of change in weight SD score from birth to 3 months, following adjustment for the covariates.

These observations accord well with those reported for children in studies that have associated the CEBQ traits with adiposity insofar as the directions of the relationships are the same (Carnell & Wardle, 2008a; Cunha et al., 2010; Gregory et al., 2010; Jahnke & Warschburger, 2008; Joyce & Zimmer-Gembeck, 2009; Parkinson et al., 2010; Sleddens et al., 2008; Viana et al., 2008; Webber et al., 2009). It is difficult to compare effect sizes

across studies as scores have rarely been standardised prior to multivariate analyses (and zero-order correlations are not usually reported), different covariates have been included, and studies with children tend to use BMI or waist SD scores while weight SD scores were used here. Nonetheless, Carnell and Wardle (2008a) reported the Pearson's correlations for 'enjoyment of food' and 'slowness in eating/ satiety responsiveness' and found relationships of a similar magnitude to those observed with infants here for both BMI SDS and waist SDS for 11-year-old children ('enjoyment of food' and BMI SDS, r=0.18, and waist SDS, r=0.20; 'satiety responsiveness/slowness in eating' and BMI SDS, r=0.22, and waist SDS, r=0.23) and for BMI SDS in 4-year-old children ('enjoyment of food', r=0.18; 'satiety responsiveness/slowness in eating', r=0.19). These findings suggest that individual differences that are present in appetitive traits from the beginning of life predict individual differences in adiposity to a modest extent, and these relationships continue to be present throughout childhood.

Fewer studies have explored the relationship between appetitive characteristics and adiposity during the milk-feeding phase due perhaps, in part, to the absence of a suitable psychometric measure prior to the BEBQ. However, two laboratory-based studies observed milk-feeding in infants and related individual differences in sucking behaviour (akin to the traits captured in the BEBQ) to variability in adiposity. Stunkard and colleagues (2004) found that faster sucking (more sucks per length of feed) at 3 months of life predicted adiposity at 1 and 2 years of age, while higher sucking pressure at 2 and 4 weeks of age was associated with greater adiposity at 1,2 and 3 years of age by Agras et al (1990). Moreover, sucking speed also differentiated infants at high risk of obesity from those at low risk of obesity (according to maternal BMI) with high risk infants taking 50% more sucks per feed than low risk infants (Stunkard et al., 1999), in concurrence with a much earlier study by Millstein (1980) who showed that high risk infants sucked more avidly in response to a sweetened solution than the low risk comparator group. The longitudinal predictive power of these characteristics for prospective weight gain, combined with their differentiation of infants at higher and lower obesity risk point towards a causal role for these appetitive traits in the development and maintenance of excess adiposity. Because Gemini is a longitudinal study it will be possible to explore the relationships between the BEBQ scales and weight prospectively over the first five years

of life. Finding that the BEBQ traits predict weight gain will contribute to the evidence-base that they sit on the causal path.

It is noteworthy that appetitive traits in relation to milk show the same graded associations across the spectrum of weight as they do for food in older children (Webber et al., 2009; Viana et al., 2008; Sleddens et al., 2008; Cunha et al., 2010; Carnell & Wardle, 2008a). All of the BEBQ scores increased in a linear fashion across the weight groups such that each subsequent category of higher weight showed more 'enjoyment of food', greater 'food responsiveness', faster eating, less 'satiety responsiveness' and a larger 'appetite size' than the neighbouring lower category, suggesting that these characteristics relate to weight across the continuum rather than distinguish the 'normal' from the 'abnormal'. It is interesting that the clinical paediatric literature has identified certain eating behaviours that are characteristic of a poorer overall appetite as key differentiators of infants who 'fail to thrive', such as being less interested in food (Wright & Birks, 2000; Wright et al., 2000), less often hungry at mealtimes (Wright & Birks, 2000; Wilensky et al., 1996; Wright et al., 2000), and less likely to enjoy mealtimes (Wright et al., 2000; Wilensky et al., 1996). While it has been established that these infants and children generally consume less energy at a given meal (or per unit of time) compared with controls (Drewett et al., 2003; Pollitt & Eichler, 1976; Parkinson et al., 2004; Whitten et al., 1969; Frank & Zeisel, 1988; Maggioni & Lifshitz, 1995), these research findings suggest that the explanation for this discrepancy may be rooted partly within appetitive differences. One study looked specifically at how appetite quality ('At present, how is your infant's appetite?' rated on 5-point scale from 'very poor' to 'very good') related to weight gain and failure to thrive over the first year of life Wright et al., 2006); appetite quality predicted weight cross-sectionally at both time-points and appetite at 6 weeks was prospectively associated with weight gain from 6 weeks to 12 months (as well as weight faltering). Collectively, these findings suggest that innate appetitive traits from the beginning of life play an important role in explaining both excessive weight gain and insufficient growth during early life.

7.5.1.2 Are these traits independently associated with weight or do they appear to share a common pathway?

When the four distinct BEBQ scales were entered into the model simultaneously (excluding 'appetite size') 'enjoyment of food' was not a significant predictor of 3-month weight or of change in weight from birth to three months, suggesting that the relationship between this characteristic and weight (or weight gain) is shared completely with one of the other eating behaviours. The effects of the other three traits were also attenuated slightly, indicating that they also overlap with one another to some extent in the way that they relate to weight, although they also have non-shared paths. This makes sense theoretically. For example, faster feeders may also compromise their responsiveness to internal cues of satiety by outpacing the natural satiety mechanisms that take a few minutes to develop, and so act in concert to encourage the development of excess weight; at the same time highly food responsive babies may override internal satiety mechanisms through attending primarily to the rewarding properties of the feeding process, and more motivated feeders feed more quickly as well.

Once 'appetite size' was added into the model, the relationship between 'food responsiveness' and 3-month weight disappeared showing that this relationship is entirely explained via 'appetite size'. On the other hand, 'slowness in eating' and 'satiety responsiveness' remained independent predictors of adiposity (although their effect sizes were further attenuated), highlighting that these two characteristics share different pathways with weight, over and above general 'appetite size', and separately from one another, to an extent. Carnell and Wardle (2008a) found that in 4 and 11-year old children 'enjoyment of food' predicted weight independently of 'satiety responsiveness/slowness in eating', suggesting that for children whose appetite is judged by consumption of a range of foods, the relationships between the different appetitive traits and weight have become more distinct; alternatively, the lack of variability in enjoyment of milk at this stage of life may limit the ability to find independent associations with weight. It is of interest that Parkinson and colleagues (2010) who included all of the CEBQ scales simultaneously with their single item measure of appetite quality (described earlier) found 'satiety responsiveness' and appetite quality measured at 5-6 years both independently predicted BMI at 7-8 years. Although these models cannot be directly compared with the models

presented here as different numbers of CEBQ scales were included in each and the scores had not been standardised, these two findings together with the results in this chapter highlight the importance of measuring 'satiety responsiveness' and 'slowness in eating' separately, and these scales should not be substituted by the stand alone item 'appetite size' when exploring relationships with weight.

'Appetite size' was the only independent appetitive predictor of change in weight from birth to three months as this was the only appetite measure to remain significant after birth weight was added in to the model along with the other BEBQ scales. This is not surprising given that birth weight is highly correlated with weight at 3 months, that the four BEBQ scales are modestly correlated with 'appetite size', that 'appetite size' has the highest correlation with 3-month weight SDS, and that three of the four BEBQ scales are significantly associated with birth weight (as shown in Chapter 6, section 6.4.4.2); after variance in 3-month weight SD score has been explained by birth weight and 'appetite size' there is little remaining variance in 3-month weight SD score, and after variance in the four BEBQ scales has been explained by 'appetite size' and birth weight there is little residual variance left to explain 3-month weight SD score.

7.5.2. Strengths and weaknesses

There are limitations to this study. Being a cross-sectional study it is not possible to draw conclusions about the directions of relationships or infer causation. The causative role that appetite plays for weight is a tricky issue that is not necessarily solved through using a prospective design – for example weight at 12 months is correlated very highly with weight at 3 months, and appetite is also correlated over time. Nevertheless, finding a prospective association between the BEBQ traits and weight later on in infancy would contribute to the evidence base. This is discussed in more detail in Chapter 12.

The sample in this study only includes twins, and it is well documented that weight and growth during infancy is different for twins and singletons (Buckler & Green, 2004; Grumbach et al., 1986). Twins are more often born prematurely which contributes to lower birth weights (Buckler & Green, 2004), and lower birth weights in general may program

catch up growth and different growth trajectories (Gluckman & Hanson, 2008; Kensara et al., 2005; Ong et al., 2000; Ong, 2006; Ong & Loos, 2006; Taylor & Poston, 2007). All analyses included both twins and took into account the clustering of twins within families. Replication of these findings in singletons would add credibility to these findings.

There was a ceiling effect for 'enjoyment of food' which may have explained the small association with weight and the fact that this particular trait did not independently predict weight (or weight change) in the context of the other three BEBQ scales. Nevertheless, although enjoyment of milk and of feeding at this young age is the norm, there was still enough variation in this trait to show a graded association with weight.

The BEBQ is a parent-report measure of appetite and as such it is possible that the associations with weight (and change in weight) arise from parents attributing higher appetitive scores to bigger infants in order to explain their body size or their growth. Behavioural validation of the BEBQ would provide confidence that the parents' reports reflect the infant's appetitive characteristics rather than parental biases. However, the BEBQ was based upon a similar parent-report measure, the CEBQ, which has been validated using behavioural measures. It is likely that parents of infants would be able to respond to the BEBQ with as much accuracy as parents of children who respond to the CEBQ. Behavioural validation of the BEBQ is under way, and this work is discussed in Chapter 12.

Feeding method (bottle-feeding and breast-feeding) showed seemingly contradictory relationships with appetite and weight during the first three months. While breast-feeding was associated with a lower weight, it was also associated with greater appetite avidity characterized by higher scores for 'food responsiveness' and 'appetite size' and lower scores for 'satiety sensitivity' (see Chapter 6, section 6.4.4.2.). These findings are somewhat surprising given that greater appetite avidity was associated with higher weight. As suggested in Chapter 6, it is possible that infants who demonstrated poorer appetites during the first few months were more likely to be bottle-fed and encouraged to gain weight by regular feeding and offering of milk, especially given that twins are generally born smaller than singletons. In the same way, infants who showed good responses to breast-feeding and gained weight at an acceptable rate may have been more likely to be breast-fed for longer. At the same time, breast-feeding and bottle-feeding could influence weight

gain independently of appetite, with bottle-fed infants on the whole growing more (and growing faster) than breast-fed infants.

Major advantages of the present analyses relate to the fact that most weight measurements were performed by health professionals (only 3.6% of data were from parents own measurements). In the UK, all children are weighed at around 2-3 months during standard health visits, in addition parents can get their infants weighed by health professionals as often as they want, and all measurements are recorded in the infant's Personal Health Record. Data on weight at 3 months were available for the majority of the sample.

A particular strength of this study is that appetitive traits akin to those of the CEBQ were associated with weight during the earliest period of life before any solid food has been introduced. Rapid weight gain in infancy is a risk factor for obesity and other diseases, and weight gained early on in life is very difficult to lose. In a review of prospective studies relating childhood weight status to adulthood obesity one-third of obese children were obese as adults, and the adult obesity risk for obese children was twice that of normal-weight children (Serdula et al., 1993). What is more, infant weight status predicts adult obesity – a large longitudinal study found that the odds ratio for being obese at 35 years of age was 2 for individuals who were obese as infants (Guo et al., 1994). Finding associations at this very early stage raises the possibility that individual differences in appetitive characteristics that are there from the beginning of life play a role in mediating growth rate.

7.5.3. Implications for theory, practice and future research

An implication of these findings is that if other researchers are interested in examining the association between appetite and weight at this age, but time and space is limited, 'slowness in eating', 'satiety responsiveness' and 'appetite size' may be measured without 'enjoyment of food' and 'food responsiveness'. However, the other scales nevertheless tap important appetite dimensions, and the separate regression analyses where they were entered separately shows that they are all related to weight; the choice of scale to measure should largely depend on the reason for appetite measurement. For example, in

the context of intervention work a focus on attenuating an infant's 'food responsiveness' may prove as fruitful as efforts to upregulate 'satiety sensitivity' in curbing overall intake to prevent excessive weight gain.

Finding individual differences in these traits at such a young age (Chapter 6), and finding associations with weight already, raises the question about where appetitive characteristics come from. There is evidence that 'enjoyment of food' and 'satiety responsiveness/slowness in eating' have a genetic basis in children (Carnell et al., 2008); moreover, sucking speed and responsiveness to sweetened solutions distinguished infants at higher or lower risk of obesity (by virtue of their parental weight status) (Millstein, 1980; Stunkard et al., 1999) pointing towards a role for these traits in mediating the intergenerational transmission of weight. Demonstrating appetite variability in early life, along with relationships with adiposity, provides two pieces of evidence that point towards a behavioural susceptibility model of weight during infancy. In order to move forward, these traits should show genetic influence in early life as well. The heritability of these traits during infancy has never been established. This is the focus of the next chapter.

Another question that has been raised by these findings is whether these appetitive traits are associated with weight because common genes influence appetite and weight, or because the same environmental factors that give rise to appetite also influence weight. We know that weight is heritable, even at this early age of three months (Beardsall et al., 2009; Dubois et al., 2007b; Gielen et al., 2008; Levine et al., 1987; Lunde et al., 2007; Pietilainen et al., 2002; Vlietinck et al., 1989; Whitfield et al., 2001); providing evidence of a genetic basis for these appetitive traits would raise the possibility that the genes that influence weight are working through appetite, giving rise to the phenotypic association. Shared pathways underlying appetite and weight are the focus of Chapter 10.

CHAPTER 8. STUDY 3: GENETIC AND ENVIRONMENTAL INFLUENCES ON APPETITIVE TRAITS IN INFANCY[62]

8.1. Background

Chapter 1 provided evidence for a range of appetitive behaviours that have been associated with weight in children and in infants; Chapter 2 illustrated that these characteristics appear to have a heritable basis in adulthood and childhood, although the heritability of these traits has never been explored during infancy. However, Stunkard and colleagues (2004) found that a faster sucking speed was the sole variable to differentiate 3-month-old infants at lower- and higher-risk of obesity as indexed according to maternal BMI values, suggesting that this behaviour may play a part in mediating the genetic transmission of weight. Likewise, Milstein (1980) found that babies with two overweight parents sucked more avidly in response to sweetened solution compared with plain water than babies with two normal-weight parents, once again raising the possibility that this trait may be an inherited behaviour involved in the actualisation of genetic weight predisposition.

An investigation into the heritability of these 'obesogenic' appetitive behaviours in early infancy is warranted in light of the evidence that these eating styles are highly heritable in children, and that preliminary evidence indicates that there may be genetic underpinnings for certain feeding styles in very young infants. Moreover, an investigation in to the origin of these weight-related traits in infancy is timely under the growing concern about the rising prevalence of childhood obesity, and the desire to understand the mechanisms involved in pathways that potentially mediate rapid weight gain early on.

The first study in Chapter 6 described the development of the Baby Eating Behaviour Questionnaire that provides a comprehensive psychometric measure of the main dimensions of infant appetite during the first three months of life, permitting for the first

[62] A version of this chapter has been published in the following paper: Llewellyn CH, van Jaarsveld CHM, Johnson L, Carnell S and Wardle J. (2010). Nature and nurture in infant appetite: analysis of the Gemini twin birth cohort. *American Journal of Clinical Nutrition*, 91, 1172-1179.

time large-scale research into the relative influences of genes and environment on these traits during infancy.

8.2. Study aims

This study assesses the relative influences of genes and environment on 'obesogenic' appetitive traits in the earliest period of life while infants are exclusively milk-fed.

8.3. Methods

8.3.1. Heritability analyses

All heritability analyses were conducted on BEBQ subscale scores that had been residualized for sex-effects and age at BEBQ completion, using a regression procedure. Because feeding problems and gestational age were associated with BEBQ scores (Chapter 6), and concordance for feeding problems was slightly higher for MZs than DZs potentially affecting the equality of environments across zygosity (Chapter 5), sensitivity analyses were conducted to ascertain whether heritability differed with and without infants with reported feeding problems, and with and without infants born before 34 weeks of age; I also checked whether heritability estimates were altered by additional adjustment for gestational age as a continuous measure (as well as age at BEBQ completion and sex). In addition, to test for parental rating biases, heritability was estimated separately for twins whose zygosity classification by the questionnaire was the same as the parental classification, and for twins whose zygosity classification by the questionnaire was different to the parental classification (finding differences could indicate that the BEBQ scores may be influenced by differential parental perception of zygosity).

If sample exclusions or additional statistical adjustment led to increases or decreases in the genetic or environmental estimates that were outside the 95% confidence interval of

the whole sample using standard adjustment only, the additional adjustment or exclusion was deemed significant. There was no evidence of genetic dominance effects or sibling interaction effects so standard ACE analyses were performed. An alpha level of 0.01 was used for the likelihood ratio test to avoid type 1 errors as a result of the large sample size.

Sex differences have sometimes been reported for eating behaviours. Boys scored significantly lower on 'satiety responsiveness' and 'slowness in eating' measured as a combined scale using the CEBQ in the full TEDS sample at age 11 (Carnell et al., 2008), and in this sample of infants males scored significantly higher on 'food responsiveness' and 'appetite size' and lower on 'satiety responsiveness' than females. In addition, genetic effects for eating behaviours often vary with sex, as highlighted in the review in Chapter 2. In particular, the heritability of 'enjoyment of food' (measured using the CEBQ) was slightly higher for males than for females in 11-year old children from the TEDS sample (Carnell et al., 2008). Sex-differences in the heritability of appetitive traits during infancy were therefore explored. Furthermore, Chapter 6 found mean differences between breast-fed and bottle-fed infants for 'food responsiveness', 'satiety responsiveness' and 'appetite size', so differences in heritability by feeding mode were also explored.

8.3.1.1. Twin correlations

Within-pair within-trait twin intraclass correlations formed the basis of the univariate heritability analyses. The correlations were calculated for all of the twins, for the sample excluding twins with any reported feeding problem, for the sample excluding twins born before 34 weeks gestation, on scores that had been additionally adjusted for gestational age, and separately for the four different sub-categories of zygosity (MZQ-MZP, MZQ-DZP, DZQ-DZP, and DZQ-MZP). Twin correlations were also calculated separately for boys and girls[63] and compared to the estimates for the sexes combined to explore sex differences in the heritability of each of the BEBQ scales. Likewise, correlations were calculated separately for pairs of twins who were both mainly bottle-fed and pairs of twins who were both mainly breast-fed in order to ascertain if estimates differed by feeding

[63] These analyses included all twins and were performed on scores that had been residualised for age at BEBQ completion and sex only.

mode, which would suggest a gene-environment interaction. Intraclass correlations were calculated using SPSS version 15 for Windows.

8.3.1.2. Covariance model-fitting

Univariate saturated models were run for all five of the appetitive traits to which the fit of subsequent ACE univariate genetic models were compared. More parsimonious sub-models were also tested against the full ACE model to assess if the additive genetic effect (A), or the shared environmental effect (C), or both (A and C) could be dropped from the full model. The LRT, AIC and BIC statistics were used to identify the most parsimonious model for each trait. As was the case with the twin correlations, the univariate models (the full ACE model and sub-models) were run for all of the twins, for the sample excluding twins with any reported feeding problem, for the sample excluding twins born before 34 weeks gestation, and on scores that had been additionally adjusted for gestational age. Parameter estimates for full ACE models were also examined for different sub-categories of MZ twins and excluding DZQ-MZPs (all DZs and MZQ-MZP; all DZs and MZQ-DZP; all MZs and DZQ-DZP) to check the estimates were of a similar magnitude[64].

A saturated sex-limited heterogeneity model was run to indicate if variances and covariances could be equated across the sexes, and full sex-limitation models were used to test for qualitative and quantitative sex-differences in the appetite scales. The LRT, AIC and BIC were used to identify the most parsimonious model for each trait. Heterogeneity models were also used to test for quantitative differences in A, C and E by feeding mode; twin pairs who were both mainly bottle-fed and twin pairs who were both mainly breast-fed were modelled as the subgroups, and these models were compared to a saturated heterogeneity model that was split by feeding mode. The same goodness-of-fit statistics were used to identify the most parsimonious model. Mx maximum-likelihood structural equation modelling software (version 32; Virginia Commonwealth University, Richmond, VA) was used to run the ACE model-fitting analyses.

[64] It was not possible to run heritability analyses using DZQ-MZPs instead of DZQ-DZPs (and all MZs) due to the very small number of DZQ-MZP pairs (n=16 pairs).

Because the residuals for 'enjoyment of food' were not normally distributed all of the univariate and sub-group analyses were repeated on this scale as a dichotomous variable split on the median of the residualized scores ($\leq$0.2982 and >0.2982), using methods for categorical data. Tetrachoric correlations were used instead of intraclass correlations; this method assumes that underlying the observed dichotomous trait is a continuously and normally distributed latent construct with a threshold distinguishing those who score high or low on the trait[65]. Tetrachoric correlations were calculated using Intercooled Stata version 9. A threshold ACE model was used instead of a standard model for continuous data; this method is based upon threshold values that may be thought of as z-scores on a theorised underlying standard normal distribution, that indicate the distribution of scores above and below the median (Neale et al., 2003b). Mx was used for the threshold analysis. Only the results from the continuous models are presented and discussed in detail in this chapter (the results from the threshold model are briefly reported in footnotes[66]) for two reasons: firstly, this allows me to compare the univariate results across the five appetitive measures more fairly; secondly, dichotomising variables results in substantial loss of variation in the data – this tends to inflate the shared environmental effect and dilutes the heritability estimate, due to more of the twins sharing the same scores. To demonstrate this effect 'slowness in eating' (for which the residuals were normally distributed) was also dichotomised and modelled using the same method – the results are shown in Appendix 5.11.

[65] Heritability may be estimated from tetrachoric correlations using the same equations as those used for the intraclass correlations.
[66] All of the analyses from the threshold models are shown in full in Appendix 5.

8.4. Results

8.4.1. Summary statistics

A total of 4623 infants had known zygosity and complete data for at least one of the BEBQ subscales. Table 8.1 shows the number of infants with known zygosity who had complete data for each subscale, by zygosity, sex and feeding method[67].

Table 8.1. Number of infants with full data for each scale of the BEBQ by zygosity, sex and feeding method

Sub-group	BEBQ Scale				
	EF	**FR**	**SE**	**SR**	**AS**
MZs	1425	1433	1439	1437	1438
DZs	3156	3154	3170	3166	3176
Boys	2264	2266	2276	2272	2281
Girls	2317	2321	2333	2331	2333
Breast-fed[1]	1446	1447	1450	1442	1447
Bottle-fed[2]	2392	2399	2414	2413	2417
All infants	4581	4587	4609	4603	4614

Abbreviations: BEBQ, Baby Eating Behaviour Questionnaire; EF, 'enjoyment of food'; FR, 'food responsiveness'; SE, 'slowness in eating'; SR, 'satiety responsiveness'; AS, 'appetite size'; MZs, monozygotic twins; DZs, dizygotic twins.

[1] 'Breast-fed' includes the number of infants from twin pairs who were mainly breast-fed with data for each BEBQ scale.

[2] 'Bottle-fed' includes the number of infants from twin pairs who were mainly bottle-fed with data for each BEBQ scale.

[67] The number of infants in the 'breast-fed' and 'bottle-fed' categories include the number of infants from pairs concordant for feeding method, rather than the number of individual twins who were mainly breast- or bottle-fed.

8.4.2. Sensitivity analyses

Intraclass correlations were very similar after infants born before 34 weeks gestational age were excluded, and following additional adjustment for gestational age, indicating that this factor did not influence heritability disproportionately. Exclusion of infants with feeding problems led to correlation coefficients that were slightly higher than the 95% confidence interval range for the whole sample with standard adjustments for MZs, but not for DZs (with the exception of 'enjoyment of food' for which the DZ correlation was also slightly higher), although the correlations were only marginally higher[68], suggesting that the effect was very small[69].

The sensitivity analyses were repeated using covariance model-fitting techniques and the estimates following additional adjustments or exclusions were again compared to the 95% confidence interval ranges of the whole sample with standard adjustments. The estimates were virtually unchanged after additional adjustment for gestational age, and after infants born before 34 weeks gestational age were excluded. Following exclusion of infants with feeding problems, there was a small increase in the heritability estimate for 'slowness in eating' estimated at 88%, increased from 84% in the full sample (95% CI: 79%-86%); the unique environmental effects were slightly lower for most BEBQ scales following exclusion of infants with feeding problems, although the differences in the estimates were very small: 'enjoyment of food' was estimated at 12%, decreased from 17% (15%-19%); 'food responsiveness' was estimated at 9%, decreased from 11% (10-13%); 'slowness in eating' was estimated at 12%, decreased from 16% (14%-17%); 'satiety responsiveness' was estimated at 11%, decreased from 16% (14%-17%)[70].

However, two points are noteworthy in relation to exclusion of infants with reported feeding problems. Firstly, the data on feeding problems includes any possible feeding problem and

[68] For 'enjoyment of food' the MZ correlation was 0.86, increased from 0.82 (95% CI: 0.80-0.85); for 'food responsiveness' the MZ correlation was 0.91, increased from 0.88 (0.86-0.89); for 'slowness in eating' the MZ correlation was 0.88, increased from 0.83 (0.81-0.85); for 'satiety responsiveness' the MZ correlation was 0.88, increased from 0.84 (0.82-0.86); for 'appetite size' the MZ correlation was 0.81, increased from 0.76 (0.73-0.79).
[69] The twin correlations for the sensitivity analyses for every BEBQ scale are shown in Appendix 5.1.
[70] The full covariance model-fitting results for the sensitivity analyses for all of the BEBQ scales are shown in Appendices 5.2 to 5.6

may include non-infant issues such as temporary disruption to feeding routine due to moving house, less significant problems such as difficulty in 'latching on' during breast-feeding, as well as more serious problems such as tube-feeding and cleft palate. Secondly, a very large number of infants had feeding problems of some sort (*n*=1831) so exclusion of all of these infants leads to a substantial reduction in the sample size, and less reliable estimates from the ACE models. In the light of the results from the sensitivity analyses, and these considerations, I felt it was not necessary to exclude infants with feeding problems. The heritability analyses reported in this chapter therefore included the whole sample with only the standard adjustment for age at BEBQ completion and sex.

Twin correlations were virtually the same for MZQ-MZPs and MZQ-DZPs for 'enjoyment of food', 'slowness in eating', 'satiety responsiveness' and 'appetite size', and the estimates for each group were within the 95% confidence interval ranges of the other; the correlations were also very similar for 'food responsiveness', although the estimate was slightly higher for MZQ-MZPs (0.90) than for the MZQ-DZPs (0.82), and sat just outside the 95% confidence interval range for the latter (0.77-0.86), although the size of the difference was very small indicating that it was unlikely to exert a large effect on heritability. The DZQ-DZP and DZQ-MZP correlations were similar for all scales although the latter had very large 95% confidence intervals due to the limited sample size, making it difficult to draw conclusions; nevertheless, in each case the DZQ-DZP correlation was within the large 95% confidence interval range for the other group[71].

Heritability estimates were virtually the same for all appetite scales for the three zygosity sub-groups tested (DZ and MZQ-MZP; DZ and MZQ-DZP; MZ and DZQ-DZP). However, the heritability estimate for 'enjoyment of food' was slightly higher for DZs and MZQ-DZPs than for the other two groups (0.87 versus 0.81), and outside the 95% confidence interval ranges, although the size difference was very small and of the all of the 95% confidence intervals overlapped suggesting that it was a trivial difference. At the same time, the heritability estimate for 'food responsiveness' was slightly lower for DZs and MZQ-DZPs than for the other groups (0.50 versus 0.61) and outside the 95% confidence intervals although again the size of the difference was small and the intervals overlapped for all of

[71] The twin correlations for the zygosity sub-groups for every BEBQ scale are shown in Appendix 5.7.

the groups. For all scales the preferred model was the same, with comparable proportions of variance being explained by additive genetic effects, and shared or unique environment effects. These findings suggest that there are no biases, or minimal biases arising from parental classification of zygosity[72].

8.4.3. Univariate findings

The intraclass correlations for the five appetitive traits are presented graphically in Figure 8.1. MZ correlations were substantially higher than DZ correlations for each subscale, which indicated a strong genetic contribution on each trait, although the size of the difference was slightly smaller for 'food responsiveness' suggesting a slightly smaller role for genetic factors for this appetitive characteristic. Moreover, the MZ correlations were very high overall indicating only a small influence of the unique environment on any trait (<25%).

The parameter estimates for the covariance model-fitting analyses are shown in Table 8.2 – for each appetitive trait the parameter estimates are shown for the full ACE model and for the three sub-models (the CE model drops the genetic component of variance, A; the AE model drops the shared environment component of variance, C; the E model drops both the genetic and shared environment components of variance, A and C). The best-fitting model for each scale is bolded (the goodness-of-fit statistics for each BEBQ scale are shown in the tables detailing the sensitivity analyses in Appendices 5.2 to 5.6[73]).

For 'food responsiveness', 'slowness in eating', 'satiety responsiveness' and 'appetite size' all three goodness-of-fit statistics were in accord that an ACE model fitted the data well compared with the saturated model; for 'enjoyment of food' the ACE model provided a worse fit to the data than the saturated model according to the likelihood ratio test and AIC, although BIC found it to be a better and more parsimonious representation of the data, indicating that an ACE model was acceptable for this trait.

[72] The parameter estimates obtained for the 5 BEBQ scales using the different samples of zygosity are shown in Appendix 5.8.
[73] The models in the top row of the tables show the goodness-of-fit statistics for the analyses presented here, which included the whole sample with standard adjustments only.

For 'enjoyment of food' the three goodness-of-fit statistics all agreed that the more parsimonious AE model fitted the data best, as no influence of the shared environment was detectable in the full ACE model (C was estimated as 0%); heritability was estimated as high at 83% with the remaining variance being explained by the unique environment. The full ACE model fitted the data best for 'food responsiveness', as dropping either the genetic or shared environment components of variance (or both) led to substantial worsening of fit according to all three criteria; in the full model heritability was estimated as moderate at 59%, and this was the only eating behaviour for which the influence of the shared environment was sizeable at 30%. 'Slowness in eating' showed a very similar pattern of heritability to 'enjoyment of food' – there was no detectable effect of the shared environment (estimated at 0%), while the genetic influence was very large at 84%, so an AE model fitted the data best[74].

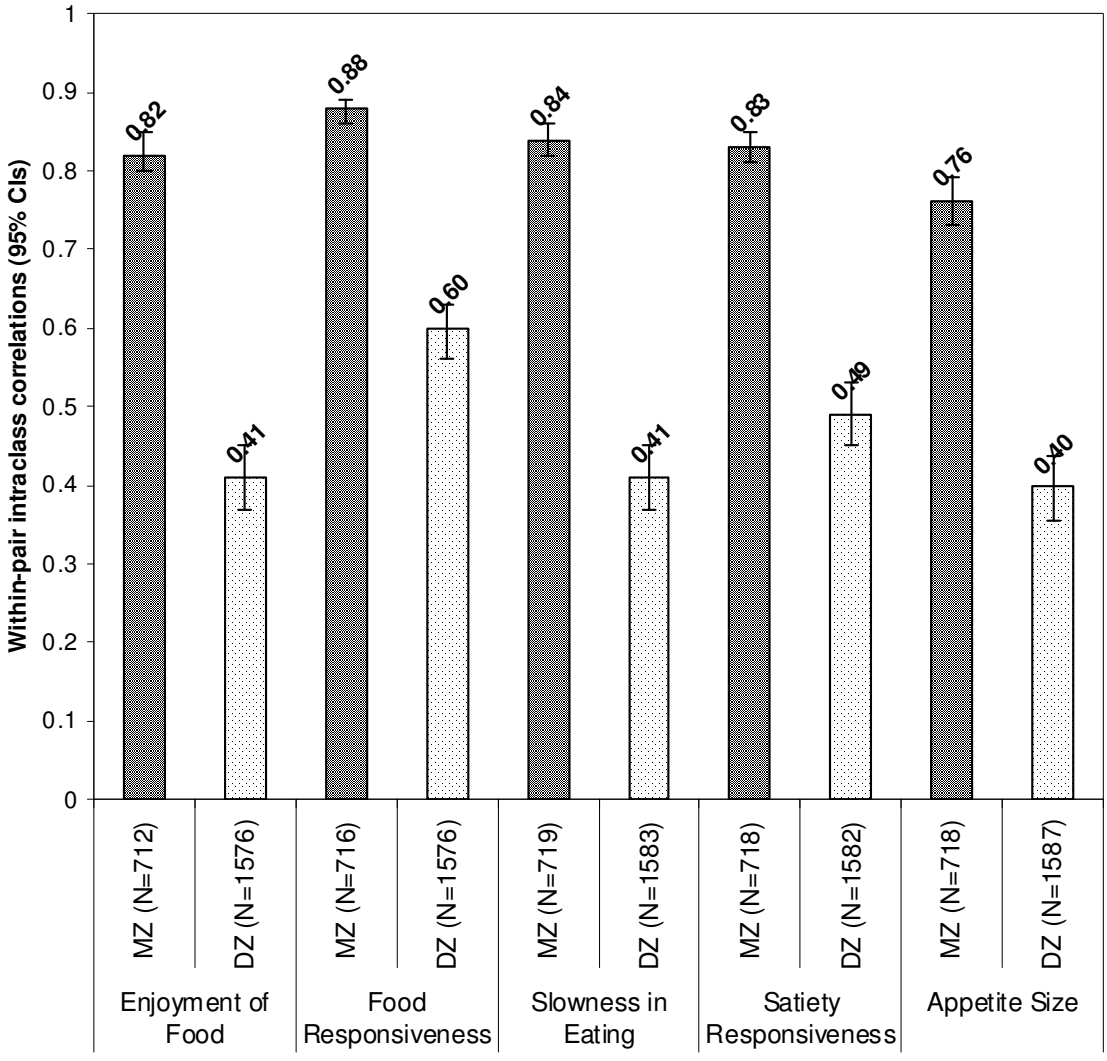
The goodness-of-fit statistics were not completely in agreement regarding the best-fitting model for 'satiety responsiveness' – the likelihood ratio test identified the full ACE model as providing the best fit, as dropping any of the parameters led to a significant worsening of fit, BIC identified the AE model as offering a more parsimonious solution to the data, and AIC indicated that the AE model had considerably less support than the full ACE model. An examination of the parameter estimates indicated that the influence of the shared environment was small (12%) but nevertheless significant in the models, so the full ACE model offered a fairer representation of the data for this eating behaviour. The ACE model estimated the heritability of 'satiety responsiveness' to be high at 72%, with the shared environment and unique environment effects playing a fairly equal role (explaining 12% and 16% of the variance respectively).

All three fit statistics were in accord that an AE model provided the best representation for the data for 'appetite size'. Genes play the most important role in influencing an infant's

[74] The results for the threshold model for 'enjoyment of food' are presented in Appendix 5.10 (the tetrachoric twin correlations are shown in Appendix 5.9). As predicted, heritability was somewhat lower when estimated using a threshold model (53%) and the effect of the shared environment was modest (45%). However, this is likely to reflect the different modeling methods rather than unreliable estimation of 'enjoyment of food' using a standard model for continuous data, because when 'slowness in eating' was modeled as a categorical variable the findings were similar to 'enjoyment of food' – heritability was much lower (66%) than the standard model, and the shared environmental effect was modest (29%) (Appendix 5.11). 'Slowness in eating' was normally distributed and had similar parameter estimates to 'enjoyment of food' in the standard model for continuous data.

overall appetite size (77%) while the unique environment explains the remaining variance (23%); the influence of the shared environment was not detectable for this trait.

Figure 8.1. Within-pair intraclass correlations (and 95% confidence intervals) for Baby Eating Behaviour Questionnaire subscale scores and 'appetite size' by zygosity



Abbreviations: MZ, monozygotic twins; DZ, dizygotic twins.

Table 8.2. Parameter estimates (95% confidence intervals) for BEBQ appetitive traits

Baby Eating Behaviour Questionnaire Scale (*n*)	Model	Additive Genetic Effect (a^2)	Shared Environment Effect (c^2)	Non-shared Environment Effect (e^2)
'Enjoyment of food' (4581)	**ACE**	0.83 (0.76-0.85)	0.00 (0.00-0.06)	0.17 (0.15-0.19)
	CE	-	0.53 (0.50-0.56)	0.47 (0.44-0.50)
	AE	**0.83 (0.81-0.85)**	**-**	**0.17 (0.15-0.19)**
	E	-	-	1.00 (1.00-1.00)
'Food responsiveness' (4587)	**ACE**	**0.59 (0.52-0.65)**	**0.30 (0.24-0.36)**	**0.11 (0.10-0.13)**
	CE	-	0.68 (0.66-0.70)	0.32 (0.30-0.34)
	AE	0.89 (0.87-0.90)	-	0.11 (0.10-0.13)
	E	-	-	1.00 (1.00-1.00)
'Slowness in eating' (4609)	**ACE**	0.84 (0.79-0.86)	0.00 (0.00-0.05)	0.16 (0.14-0.17)
	CE	-	0.54 (0.51-0.57)	0.46 (0.43-0.49)
	AE	**0.84 (0.83-0.86)**	**-**	**0.16 (0.14-0.17)**
	E	-	-	1.00 (1.00-1.00)
'Satiety responsiveness' (4603)	**ACE**	**0.72 (0.65-0.80)**	**0.12 (0.05-0.19)**	**0.16 (0.14-0.17)**
	CE	-	0.59 (0.56-0.62)	0.41 (0.38-0.44)
	AE	0.85 (0.83-0.86)	-	0.15 (0.14-0.17)
	E	-	-	1.00 (1.00-1.00)
'Appetite size' (4614)	**ACE**	0.73 (0.64-0.79)	0.03 (0.00-0.11)	0.23 (0.21-0.26)
	CE	-	0.51 (0.48-0.54)	0.49 (0.46-0.52)
	AE	**0.77 (0.74-0.79)**	**-**	**0.23 (0.21-0.26)**
	E	-	-	1.00 (1.00-1.00)

Model: CE, AE and E models are nested within the full ACE model. The ACE model dissects the phenotypic variance into a^2, c^2 and e^2; the CE model drops the a^2 parameter and assesses the variance explained by the c^2 and e^2 parameters only; the AE model drops the c^2 parameter and assesses the variance explained by the a^2 and e^2 parameters only; the E model drops both the a^2 and c^2 parameters and assesses the variance explained by e^2 only. The best-fitting and most parsimonious model for each BEBQ scale is bolded.

8.4.4. Sub-group findings

8.4.4.1 Sex differences in genetic and environmental influences on appetitive traits

8.4.4.1.1. Twin correlations

The twin correlations for each of the BEBQ subscales are presented graphically in Figure 8.2 below for the different sex categories of MZ and DZ twins. As can be seen, for all the appetitive traits the male and female MZ correlations were much higher than the male, female and opposite-sex DZ correlations, indicating a strong genetic contribution for the traits on males and females. There did not appear to be any quantitative sex-differences in heritability for any of the appetitive traits as all of the 95% confidence intervals overlapped for same-sex male and female pairs within MZs and DZs. In addition, for 'food responsiveness', 'slowness in eating', 'satiety responsiveness' and 'appetite size' the opposite-sex DZs did not differ significantly in their correlations compared with the same-sex DZs indicating that there are no qualitative sex differences involved in the genetic influences on these traits. The DZ correlation for 'enjoyment of food' was significantly lower than the same-sex DZ correlations as the 95% confidence intervals did not overlap[75], suggestive of some qualitative differences between males and females in the genetic or environmental influences on this trait, although the correlation was not substantially lower.

8.4.4.1.2. Covariance model-fitting analyses

The parameter estimates for the sex-limitation covariance model-fitting analyses are shown in Tables 8.3. to 8.7 for the different BEBQ scales. The goodness-of-fit statistics for the sex-limitation models for each scale are shown in Appendix 5.12. The full sex-limitation model with r_A free allows the additive genetic correlation (r_A) to vary between 0 and 0.5 for opposite-sex DZs whilst remaining fixed at 0.5 for same-sex DZs (as well as allowing estimates of A, C and E to differ for males and females) to test for qualitative sex-differences in the genetic influence on the appetitive traits, while keeping the shared

[75] Female DZs = 0.48 (0.40, 0.55), male DZs = 0.50 (0.42, 0.57); opposite-sex DZs = 0.33 (0.27, 0.39).

environment correlation fixed at 1.0 for both opposite-sex and same-sex DZs (and MZs). The full sex-limitation model with r_C free allows the shared environment correlation (r_C) to vary between 0 and 1.0 for opposite-sex DZs whilst remaining fixed at 1.0 for same-sex DZs (as well as allowing quantitative differences in A, C and E across males and females) to test for qualitative sex-differences in the shared environmental influences on the traits, while keeping the additive genetic correlation fixed at 0.5 for both opposite-sex and same-sex DZs. The common effects model fixes the additive genetic and shared environment correlations between the opposite-sex DZs to be the same as those between same-sex DZs (r_A=0.5; r_C=1.0), but allows for quantitative differences in the effect sizes of A, C and E across males and females. Finally, the null model constrains all parameters to be the same for males and females, not allowing sex differences of any kind.

Enjoyment of Food

For 'enjoyment of food', the likelihood ratio test indicated that there are quantitative sex-differences in the effect sizes of A, C and E because equating these parameters across males and females (the null model) led to a significant worsening of fit compared to the common effects model. AIC also preferred the common effects model. On the other hand, BIC indicated that it was better to combine the sexes in a null model. The 95% confidence intervals for A and C did not overlap for males and females in the common-effects model suggesting that this was the fairer model for the data (Table 8.3). According to the common-effects model heritability was higher for males than females (81% versus 62%) and the 95% confidence intervals did not overlap for the shared environment parameter either, indicating that this was modest for females (22%) but not detectable for males (0%). The unique environment effect was similar for both sexes (19% and 16% for males and females respectively)[76].

[76] The results for the threshold sex-limitation covariance model-fitting for 'enjoyment of food' are shown in Appendices 5.14 (goodness-of-fit statistics) and 5.15 (parameter esimates); the tetrachoric twin correlations by sex and zygosity are shown in Appendix 5.13.. A null model fitted the data best according to all three fit statistics, probably as a result of reduced power to detect interaction effects with categorical methods.

Food Responsiveness

All three goodness-of-fit statistics favoured the common effects model for 'food responsiveness' that permitted A, C and E to differ for males and females. However, all of the 95% confidence intervals overlapped for the A, C and E parameters, and the male estimates sat within the female 95% confidence intervals and vice versa (Table 8.4), indicating that any differences were marginal. Heritability was similar for males and females (59% and 52% respectively) and in keeping with the univariate estimate, and the shared environment appeared to play an important role for both sexes (30% for males and 35% for females).

Slowness in Eating

A null model was identified by all the fit statistics as the preferred model for 'slowness in eating' that did not allow sex differences in any parameters. The estimates were therefore the same as the univariate analyses – heritability was high at 84% with the unique environment explaining the remaining variation (Table 8.5).

Satiety Responsiveness

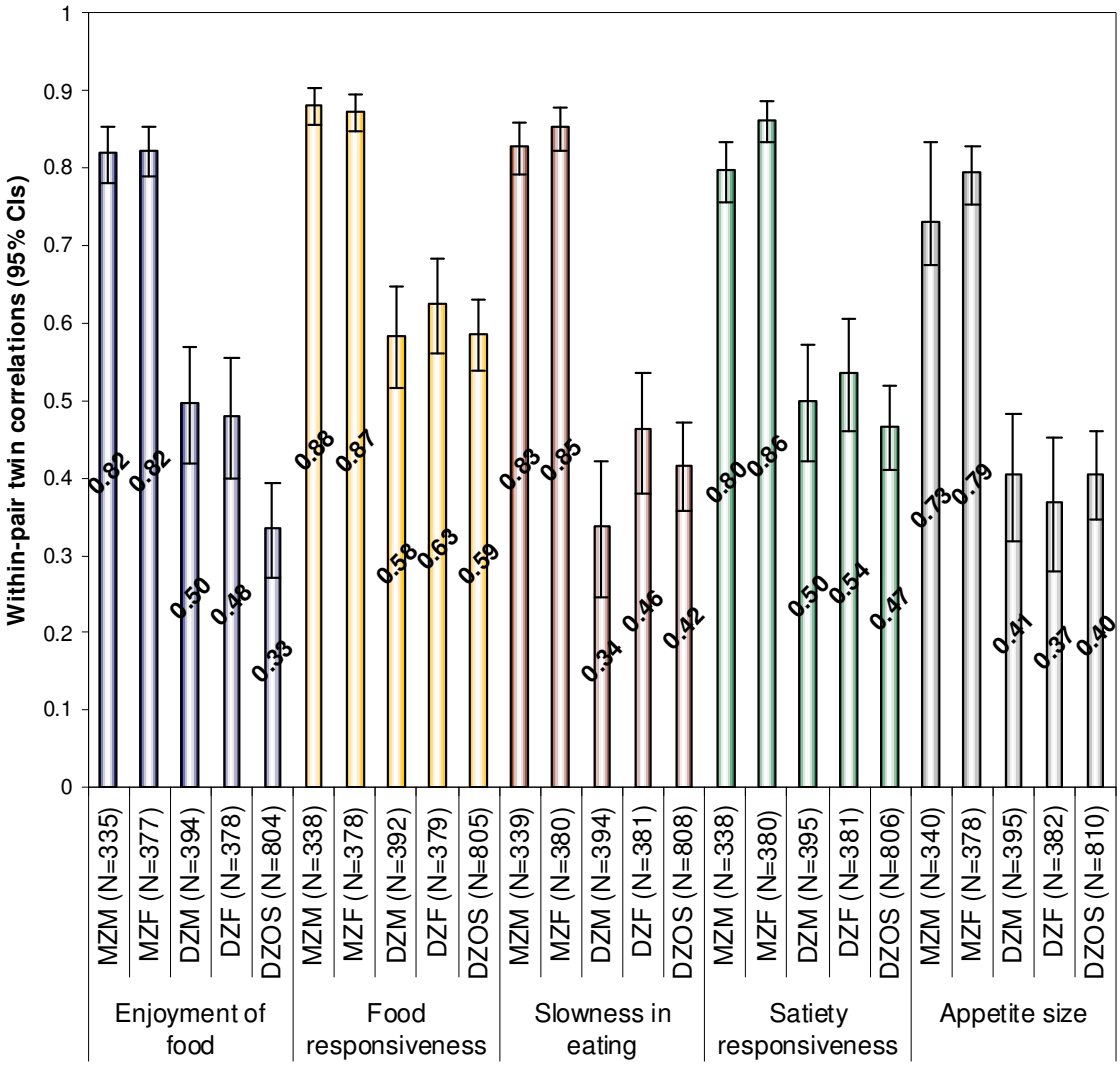
For 'satiety responsiveness' the likelihood ratio test and AIC favoured a common effects model, but BIC preferred a null model that constrained all of the parameters to be the same for males and females. All of the 95% confidence intervals overlapped for SR, and the male estimates sat within the females 95% confidence intervals and vice versa, suggesting that the parameters could be combined in a null model (Table 8.6), with estimates being the same as those from the univariate analyses – modest heritability at 72%, and similar influences from the shared environment and unique environment.

Appetite Size

The likelihood ratio test and AIC indicated that it was better to model separate A, C and E estimates for males and females in a common effects model, but BIC preferred to combine the sexes in a null model. The parameter estimates indicated that it was, indeed acceptable to combine the sexes as the male and female estimates sat within one another's 95% confidence intervals (Table 8.7). The estimates were therefore in keeping

with the univariate findings of high heritability (73%) and most of the remaining influence coming from the effects of the unique child environment.

Figure 8.2. Within-pair intraclass correlations (and 95% confidence intervals) for Baby Eating Behaviour Questionnaire subscale scores by all combinations of zygosity and sex



Abbreviations: MZM, monozygotic male twins; MZF, monozygotic female twins; DZM, dizygotic male twins; DZF, dizygotic female twins; DZOS, dizygotic opposite-sex twins.

Table 8.3. Parameter estimates (95% confidence intervals) for sex-limitation model-fitting for 'enjoyment of food'

Model	Male parameter estimates			Female parameter estimates			r_A	r_C
	a^2_m	c^2_m	e^2_m	a^2_f	c^2_f	e^2_f		
Full sex-limitation model (r_A free)	0.67 (0.54-0.82)	0.14 (0.00-0.27)	0.19 (0.16-0.22)	0.67 (0.53-0.83)	0.18 (0.02-0.31)	0.15 (0.13-0.18)	0.26 (0.11-0.48)	1.00
Full sex-limitation model (r_C free)	0.81 (0.47-0.84)	0.00 (0.00-0.32)	0.19 (0.16-0.22)	0.62 (0.47-0.74)	0.22 (0.11-0.37)	0.16 (0.13-0.18)	0.50	0.61 (0.00-1.00)
Common effects model	**0.81 (0.78-0.84)**	**0.00 (0.00-0.01)**	**0.19 (0.16-0.22)**	**0.62 (0.51-0.74)**	**0.22 (0.11-0.33)**	**0.16 (0.13-0.18)**	**0.50**	**1.00**
	Parameter estimates for sexes combined							
	a^2		c^2		e^2			
Null model	0.83 (0.76-0.85)		0.00 (0.00-0.06)		0.17 (0.15-0.19)		0.50	1.00

Abbreviations: a^2_m, c^2_m, e^2_m, additive genetic, shared environmental and non-shared environmental estimates for males, respectively; a^2_f, c^2_f, e^2_f, additive genetic, shared environmental and non-shared environmental estimates for females, respectively; a^2, c^2, e^2, additive genetic, shared environmental and non-shared environmental estimates respectively for males and females combined; r_A, genetic correlation between opposite-sex dizygotic twin pairs; r_C, shared environmental correlation between opposite-sex dizygotic twin pairs.

Models (the best-fitting model is bolded):
Full Sex-Limitation Model (r_A free) – the additive genetic correlation (r_A) is estimated freely but the shared environment correlation (r_C) is fixed at 1.00 for opposite-sex dizygotic twin pairs, and ACE parameters are estimated separately for males and females.
Full Sex-Limitation Model (r_C free) – the shared environment correlation (r_C) is estimated freely but the additive genetic correlation (r_A) is fixed at 0.5 for opposite-sex dizygotic twin pairs, and ACE parameters are estimated separately for males and females.
Common Effects Model – r_A is fixed at 0.5 and r_C is fixed at 1.00 for opposite-sex dizygotic twin pairs, and ACE parameters are estimated separately for males and females.
Null Model – all parameter estimates are equated for males and females.

Table 8.4. Parameter estimates (95% confidence intervals) for sex-limitation model-fitting for 'food responsiveness'

Model	Male parameter estimates			Female parameter estimates			r_A	r_C
	a^2_m	c^2_m	e^2_m	a^2_f	c^2_f	e^2_f		
Full sex-limitation model (r_A free)	0.56 (0.45-0.67)	0.33 (0.22-0.44)	0.11 (0.09-0.13)	0.50 (0.40-0.62)	0.37 (0.25-0.47)	0.13 (0.11-0.15)	0.46 (0.34-0.50)	1.00
Full sex-limitation model (r_C free)	0.56 (0.45-0.67)	0.33 (0.22-0.44)	0.11 (0.09-0.13)	0.50 (0.40-0.62)	0.37 (0.25-0.47)	0.13 (0.11-0.15)	0.50	0.94 (0.81-1.00)
Common effects model	**0.59 (0.48-0.68)**	**0.30 (0.21-0.41)**	**0.11 (0.09-0.13)**	**0.52 (0.41-0.63)**	**0.35 (0.25-0.46)**	**0.13 (0.11-0.15)**	**0.50**	**1.00**
	Parameter estimates for sexes combined							
	a^2		c^2		e^2			
Null model	0.59 (0.52-0.65)		0.30 (0.24-0.36)		0.11 (0.10-0.13)		0.50	1.00

See footnotes for Table 8.3.

Table 8.5. Parameter estimates (95% confidence intervals) for sex-limitation model-fitting for 'slowness in eating'

Model	Male parameter estimates			Female parameter estimates			r_A	r_C
	a^2_m	c^2_m	e^2_m	a^2_f	c^2_f	e^2_f		
Full sex-limitation model (r_A free)	0.82 (0.74-0.85)	0.00 (0.00-0.00)	0.18 (0.15-0.21)	0.80 (0.66-0.88)	0.07 (0.00-0.20)	0.13 (0.12-0.16)	0.50 (0.40-0.50)	1.00
Full sex-limitation model (r_C free)	0.82 (0.74-0.85)	0.00 (0.00-0.00)	0.18 (0.15-0.21)	0.80 (0.66-0.88)	0.07 (0.00-0.20)	0.13 (0.12-0.16)	0.50	1.00 (0.00-1.00)
Common effects model	0.82 (0.74-0.85)	0.00 (0.00-0.00)	0.18 (0.15-0.21)	0.80 (0.66-0.88)	0.07 (0.00-0.20)	0.13 (0.12-0.16)	0.50	1.00
	Parameter estimates for sexes combined							
	a^2		c^2		e^2			
Null model	**0.84 (0.79-0.86)**		**0.00 (0.00-0.05)**		**0.16 (0.14-0.17)**		**0.50**	**1.00**

See footnotes for Table 8.3.

Table 8.6. Parameter estimates (95% confidence intervals) for sex-limitation model-fitting for 'satiety responsiveness'

Model	Male parameter estimates			Female parameter estimates			r_A	r_C
	a^2_m	c^2_m	e^2_m	a^2_f	c^2_f	e^2_f		
Full sex-limitation model (r_A free)	0.65 (0.51-0.78)	0.16 (0.03-0.29)	0.19 (0.16-0.23)	0.65 (0.52-0.79)	0.23 (0.09-0.35)	0.12 (0.10-0.14)	0.42 (0.30-0.50)	1.00
Full sex-limitation model (r_C free)	0.65 (0.51-0.78)	0.16 (0.03-0.29)	0.19 (0.16-0.23)	0.65 (0.52-0.79)	0.23 (0.09-0.35)	0.12 (0.10-0.14)	0.50	0.74 (0.47-1.00)
Common effects model	0.73 (0.56-0.80)	0.08 (0.02-0.24)	0.19 (0.12-0.23)	0.65 (0.52-0.82)	0.23 (0.06-0.35)	0.12 (0.10-0.19)	0.50	1.00
	Parameter estimates for sexes combined							
	a^2		c^2		e^2			
Null model	**0.72 (0.65-0.80)**		**0.12 (0.05-0.19)**		**0.16 (0.14-0.17)**		**0.50**	**1.00**

See footnotes for Table 8.3.

Table 8.7. Parameter estimates (95% confidence intervals) for sex-limitation model-fitting for 'appetite size'

Model	Male parameter estimates			Female parameter estimates			r_A	r_C
	a^2_m	c^2_m	e^2_m	a^2_f	c^2_f	e^2_f		
Full sex-limitation model (r_A free)	0.66 (0.50-0.78)	0.09 (0.00-0.24)	0.25 (0.22-0.30)	0.76 (0.63-0.81)	0.03 (0.00-0.15)	0.21 (0.18-0.25)	0.50 (0.39-0.50)	1.00
Full sex-limitation model (r_C free)	0.66 (0.50-0.78)	0.09 (0.00-0.24)	0.25 (0.22-0.30)	0.76 (0.63-0.81)	0.03 (0.00-0.15)	0.21 (0.18-0.25)	0.50	1.00 (0.00-1.00)
Common effects model	0.66 (0.50-0.78)	0.09 (0.00-0.24)	0.25 (0.21-0.30)	0.76 (0.63-0.81)	0.03 (0.00-0.15)	0.21 (0.18-0.25)	0.50	1.00
	Parameter estimates for sexes combined							
	a^2		c^2		e^2			
Null model	**0.73 (0.64-0.79)**		**0.03 (0.00-0.11)**		**0.23 (0.21-0.26)**		**0.50**	**1.00**

See footnotes for Table 8.3.

8.4.4.2. Differences in genetic and environmental influences on appetitive traits by feeding method

8.4.4.2.1. Twin correlations

The twin correlations for each of the BEBQ subscales are presented graphically in Figure 8.3 below for the different feeding method categories of MZ and DZ twins. For all of the scales the bottle-fed and breast-fed MZ correlations were much higher than the bottle-fed and breast-fed DZ correlations, indicating a strong genetic contribution for bottle-fed and breast-fed infants on each trait. However, there appeared to be some differences in the size of the correlations between bottle-fed and breast-fed DZs for 'food responsiveness', 'slowness in eating' and 'satiety responsiveness'; for these traits the bottle-fed DZs had significantly lower correlations than the breast-fed DZs as the 95% confidence intervals did not overlap[77], suggesting that heritability may be higher for bottle-fed than breast-fed infants. No such effect appeared to be present for 'enjoyment of food' or 'appetite size'.

8.4.4.2.2. Covariance model-fitting analyses

The parameter estimates for each of the BEBQ traits for the feeding method interaction models are shown in Table 8.8. The goodness-of-fit statistics for the different models for each scale are shown in Appendix 5.16. The results from the covariance model-fitting were largely in accord with the twin correlations; they are discussed below. The common effects model allows A, C and E to differ for bottle-fed and breast-fed infants, while a null model constrains all of the parameters to be the same for both feeding methods.

Enjoyment of food

The likelihood ratio test and AIC preferred the common effects model for 'enjoyment of food' that allowed different A, C and E estimates for bottle-fed and breast-fed infants, but BIC favoured combining the feeding methods in a null model. All of the 95% confidence

[77] FR: bottle fed DZs = 0.54 (0.49, 0.59), breast-fed DZs = 0.66 (0.60, 0.70); SE: bottle-fed DZs = 0.36 (0.29, 0.41), breast-fed DZs = 0.56 (0.49, 0.61); SR: bottle-fed DZs = 0.46 (0.41, 0.51), breast-fed DZs = 0.58 (0.52, 0.64).

intervals overlapped for A, C and E so a null model provided the most parsimonious representation of the data. (Table 8.8) The results from the null model were in keeping with the univariate findings that heritability is high for all infants (83%) and the unique environment effect explains the remaining variance[78].

Food responsiveness

A common effects model was identified by the likelihood ratio test and AIC as providing the best-fit for 'food responsiveness', while BIC again preferred the null model. Inspection of the parameter estimates from the model indicated that a common effects model was probably fairer for this eating behaviour as the 95% confidence intervals did not overlap for the unique environment effect that was slightly lower for the breast-fed infants (8% versus 13%), the heritability estimate for the breast-fed infants was lower than the bottle-fed infants' (55% versus 66%) and outside the confidence interval for the bottle-fed group and vice versa, while the shared environment effect was higher for the breast-fed infants than the bottle-fed infants (37% versus 20%) and again was outside the 95% confidence interval of the bottle-fed group and vice versa (Table 8.8).

Slowness in eating

All of the goodness-of-fit statistics favoured the common effects model for 'slowness in eating'. For this trait, breast-fed infants had a significantly lower heritability estimate than the bottle-fed infants (64% versus 83%) as the 95% confidence intervals did not overlap, and the shared environment effect was significantly higher for breast-fed infants than for bottle-fed infants (24% versus 0%) also indicated by confidence intervals that did not overlap (Table 8.8).

Satiety responsiveness

For 'satiety responsiveness' the likelihood ratio test and AIC indicated that a common effects model fitted the data better, that allowed for quantitative differences in A, C and E

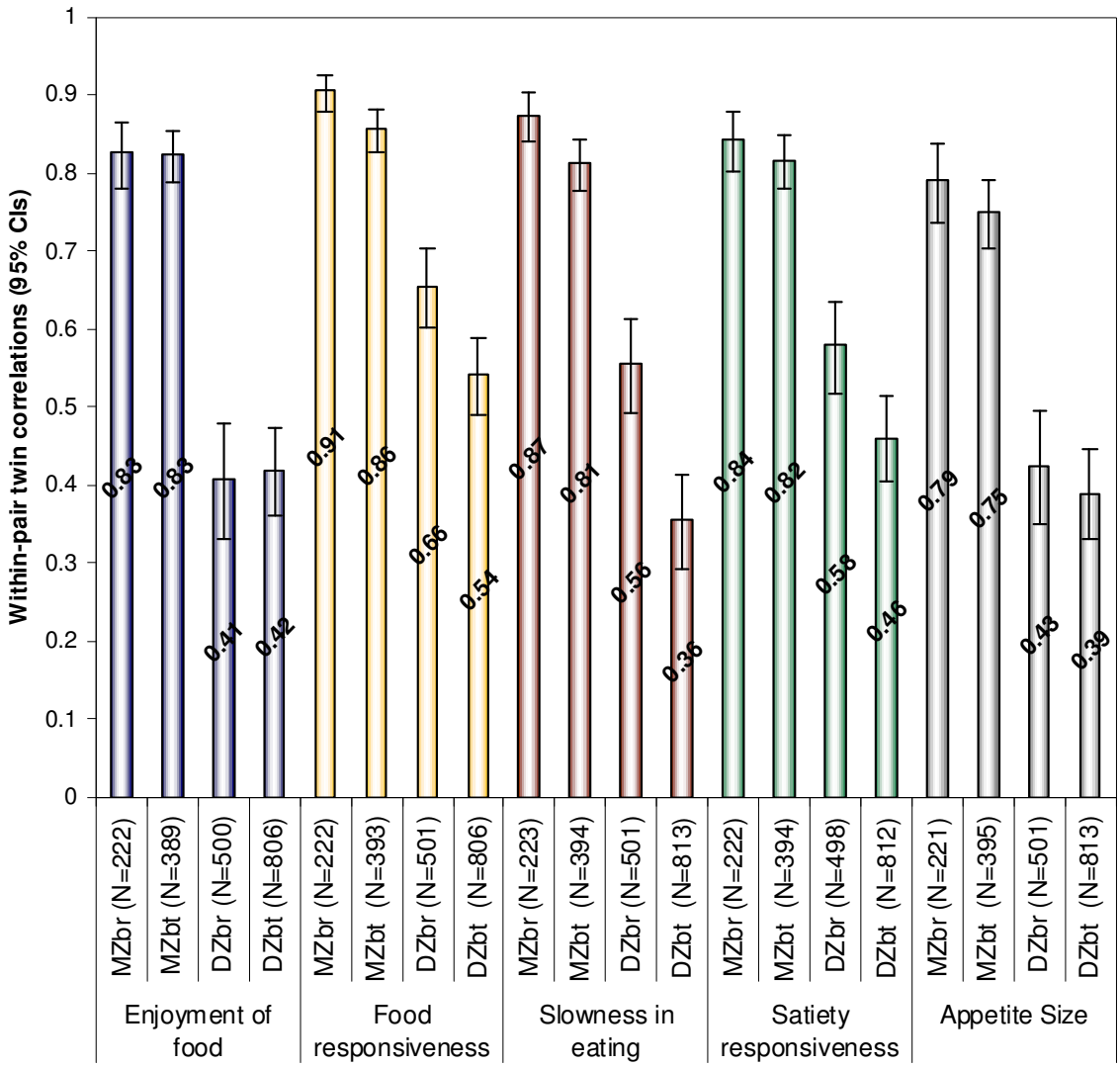
[78] The results of the threshold model for 'enjoyment of food' by feeding method are shown in Appendices 5.18 (goodness-of-fit statistics) and 5.19 (parameter estimates); the tetrachoric twin correlations by feeding method and zygosity are shown in Appendix 5.17. A null model was also preferred when 'enjoyment of food' was modeled as a categorical variable.

for bottle and breast-fed infants; BIC did not clearly favour one model over the other. The breast-fed heritability estimate was somewhat lower than the bottle-fed estimate (62% versus 78%) and each sat outside the 95% confidence interval of the other, and conversely the shared environment effect was somewhat higher for breast-fed infants than for bottle-fed infants (25% versus 6%), and again each group estimate was outside the 95% confidence interval for the other group, indicating that a common effects model that allowed the parameters to be estimated separately may fit the data slightly better (Table 8.8).

Appetite Size

The three goodness-of-fit statistics were in accord that there was no gene-environment interaction with feeding method for ‘appetite size’. The best-fitting model combined the feeding methods.

Figure 8.3. Within-pair intraclass correlations (and 95% confidence intervals) for Baby Eating Behaviour Questionnaire subscale scores by all combinations of zygosity and feeding method



Abbreviations: MZbr, breast-fed monozygotic twins; MZbt, bottle-fed monozygotic twins; DZbr, breast-fed dizygotic twins; DZbt, bottle-fed dizygotic twins.

Table 8.8. Parameter estimates (95% confidence intervals) for feeding method interaction model-fitting for BEBQ subscales

BEBQ Scale	Common Effects Model						Null Model		
	Breast-feeding parameter estimates			Bottle-feeding parameter estimates			Feeding methods combined		
	a^2_{br}	c^2_{br}	e^2_{br}	a^2_{bt}	c^2_{bt}	e^2_{bt}	a^2	c^2	e^2
EF	0.84 (0.72-0.87)	0.00 (0.00-0.11)	0.16 (0.13-0.20)	0.82 (0.71-0.85)	0.01 (0.00-0.11)	0.18 (0.15-0.20)	**0.83 (0.76-0.85)**	**0.00 (0.00-0.06)**	**0.17 (0.15-0.19)**
FR	**0.55 (0.46-0.65)**	**0.37 (0.27-0.46)**	**0.08 (0.07-0.10)**	**0.66 (0.57-0.76)**	**0.20 (0.10-0.29)**	**0.13 (0.11-0.16)**	0.61 (0.55-0.69)	0.27 (0.20-0.34)	0.11 (0.10-0.13)
SE	**0.64 (0.52-0.76)**	**0.24 (0.11-0.35)**	**0.12 (0.10-0.16)**	**0.83 (0.78-0.85)**	**0.00 (0.00-0.00)**	**0.17 (0.15-0.20)**	0.84 (0.76-0.86)	0.00 (0.00-0.00)	0.16 (0.14-0.18)
SR	**0.62 (0.51-0.74)**	**0.25 (0.13-0.36)**	**0.13 (0.10-0.16)**	**0.78 (0.67-0.86)**	**0.06 (0.00-0.16)**	**0.16 (0.14-0.19)**	0.72 (0.64-0.80)	0.13 (0.05-0.20)	0.15 (0.13-0.17)
AS	0.71 (0.56-0.82)	0.07 (0.00-0.21)	0.21 (0.18-0.26)	0.74 (0.62-0.79)	0.02 (0.00-0.13)	0.24 (0.21-0.28)	**0.73 (0.63-0.79)**	**0.03 (0.00-0.12)**	**0.23 (0.21-0.26)**

Abbreviations: BEBQ, Baby Eating Behaviour Questionnaire; EF, 'enjoyment of food; FR, 'food responsiveness'; SE, 'slowness in eating'; SR, 'satiety responsiveness'; AS, 'appetite size'; a^2_{br}, c^2_{br}, e^2_{br}, additive genetic, shared environmental and non-shared environmental estimates respectively for breast-fed infants; a^2_{bt}, c^2_{bt}, e^2_{bt}, additive genetic, shared environmental and non-shared environmental estimates respectively for bottle-fed infants; a^2, c^2, e^2, additive genetic, shared environmental and non-shared environmental estimates respectively for bottle-fed and breast-fed infants combined.

Models (the best-fitting model is bolded):
Common Effects Model – ACE parameters are estimated separately for breast-fed and bottle-fed infants.
Null Model – all parameter estimates are equated for breast-fed and bottle-fed infants.

8.5. Discussion

8.5.1. Summary of univariate findings

As far as I am aware, this is the first study to investigate the heritability of appetitive traits in the first 3 months of life, during the period of exclusive milk-feeding. Substantial heritability was found for all five appetitive characteristics – the genetic effect was large for 'enjoyment of food' (83%), 'slowness in eating' (84%), 'satiety responsiveness' (72%) and 'appetite size', (73%) and moderate for 'food responsiveness' (59%). These novel results suggest that genes are playing an important role in appetite regulation from the earliest possible period of feeding, before any variation in food type has been introduced.

The genetic influence on 'enjoyment of food' during this early period of life was very strong (83%), in keeping with the estimate observed in 11-year old twins from TEDS (75%) (Carnell et al., 2008). What is most surprising is that in the first few months of life 'enjoyment of food' shows a comparable level of genetic influence to age 11 despite no variety of food choice in the early milk-feeding period.

The estimate for 'slowness in eating' was as high as that for 'enjoyment of food' (84%). Such high genetic influence on this trait suggests that the speed at which an infant feeds may provide a sensitive measure of raw appetitive drive, and a window into the genetic influence on motivation to eat at this young age. This particular feeding behaviour has shown robust associations with adiposity in early life (Agras et al., 1990; Stunkard et al., 2004) and is able to distinguish between infants at lower and higher risk of obesity based on maternal body mass index (Stunkard et al., 2004). These findings, combined with the growing evidence of the genetic influence on weight and growth rate in early life (Cai et al., 2007; Demerath et al., 2007; van Dommelen et al., 2004), support the theory that feeding rate may be one of the behaviours that mediates the genetically determined growth rate in early infancy. This early feeding trait may be a precursor of eating speed, which was recently found to be heritable when measured behaviourally in 11-year old twins, although to a slightly lower degree (62%) (Llewellyn et al., 2008).

The estimate for 'satiety responsiveness' in infants (72%) is also similar to the estimate of 63% reported by Carnell et al (2008) for 11 year olds, which indicates that genes play an important role in the regulation of satiety sensitivity from the beginning of life and may continue to regulate these traits later on in childhood. 'Food responsiveness' showed only a moderate genetic influence (59%). The 'familiality' of this trait has been examined behaviourally in 4-19 year old Hispanic siblings using the 'eating in the absence of hunger' paradigm, and the estimate they reported (51%) was in keeping with these results for the milk-feeding situation (Fisher et al., 2007). These findings suggest that the genetic influence on this trait in the early developmental years may be fairly consistent. In adults the 'Disinhibition' scale of the TFEQ and the 'externality' scale of the DEBQ have been used to measure responsiveness to external cues of food but heritability and 'familiality' estimates have ranged from 0% to 69% across different samples (de Castro & Lilenfeld, 2005; Keskitalo et al., 2008; Neale et al., 2003a; Provencher et al., 2005; Steinle et al., 2002; Sung et al., 2010; Tholin et al., 2005). It is possible that, in adult life, the influence of social and psychological factors such as self-regulation modify the expression of the genetic effect. 'Appetite size' was highly heritable too at 73%, suggesting that individual differences in appetite avidity in a general sense are largely determined by individual differences in genetics from the beginning of life, in keeping with the other traits.

Finding such high genetic influences on these traits suggests that it is worthwhile looking for specific genetic variants that may be influencing appetite at this young age. The search for common variants associated with appetite has already begun. Of particular interest is the fat mass and obesity associated gene (*FTO*) on human chromosome 16, the first common variant found to relate to adiposity in adults and children (Loos & Bouchard, 2008; Dina et al., 2007; Cornes et al., 2009; Chang et al., 2008; Cha et al., 2008; Frayling et al., 2007). Those who carry two copies the of high risk allele (approximately 16% of the population) are on average 3kg heavier than non-carriers (Frayling et al., 2007). *FTO* is highly expressed in areas of the hypothalamus associated with feeding (Stratigopoulos et al., 2008), expression of it varies with acute food deprivation (Gerken et al., 2007), inhibition of *FTO* expression in the arcuate nucleus of the hypothalamus of rats leads to increased food intake but over-expression reduces it (Tung et al., 2010), and mice bred with additional copies of *FTO* have increased food intake, are obese, and have lower leptin levels (Church et al., 2010). Together, this research base suggests that one of the

pathways through which *FTO* influences body weight is likely to be appetite, and this has led to a number of studies exploring its effect on eating behaviour.

Using an energy compensation paradigm with 76 Scottish children aged 4-10 years Cecil and colleagues (2008) found that the higher risk allele was associated with increased energy intake in the no-energy preload condition and the low-energy preload condition, with the same trend for the high-energy preload condition, (although it did not reach statistical significance here, perhaps due to limited statistical power), but did not appear to be involved in the regulation of energy expenditure. Wardle and colleagues investigated the relationship between eating behaviours and *FTO* variants in two studies with children from the TEDS sample (Wardle et al., 2008b; Wardle et al., 2009). Using the EAH paradigm with a subsample of 131 children aged 4-5 years, carriers of the higher risk allele were shown to eat significantly more than children homozygous for the low risk allele (Wardle et al., 2009). In another study the relationship between 'satiety responsiveness' (measured using the CEBQ) and *FTO* variants was explored in the full TEDS sample (*n*=3337) when they were aged 8-11 years (Wardle et al., 2008b); children homozygous for the higher risk allele scored significantly lower on the scale, indicating lower sensitivity to satiety. *FTO* has also been shown to influence energy intake in adults and children (Speakman et al., 2008; Timpson et al., 2008). Variants near the MC4R gene have also been related to increased 'hunger' scores measured using the TFEQ, higher energy intake, greater fat intake and increased snacking (Cole et al., 2010; Qi et al., 2008; Stutzmann et al., 2009). The effects of *FTO* and MC4R on infant appetite have never been explored. DNA has been collected on the Gemini sample and a number of potential variants will be explored to better understand the role of specific genes during this early period of life.

The shared environment effect differed substantially between traits. No effect was found for 'enjoyment of food', 'slowness in eating' or 'appetite size' indicating either that the speed with which an infant consumes milk, their perceived enjoyment of the feeding interaction, and their appetite size in general may not be influenced by any factors that the two twins share in common, or that the effect of the shared environment was too small to detect with the available sample size. In keeping with this we found no evidence of an influence of the shared environment for eating rate measured behaviourally in 11-year old twin children from the TEDS subsample (Llewellyn et al., 2008), although Carnell and

colleagues (2008) found a small but significant effect at age 11 (10%) in the full TEDS sample. These findings suggest that the role of the shared environment is minimal on these traits, even during early life. For these two traits it could be the case that in this early period of life, the majority of the influence on these behaviours comes from genes and that the residual non-shared environment effect reflects a fair amount of random error of measurement, as the Cronbach's alphas indicated that about 20% of the variance in these scales are due to error.

In contrast, a moderate shared environment effect of 30% was found for 'food responsiveness' which suggests that shared aspects of the intra-uterine or subsequent rearing environment influenced the twins' appetitive responses to milk and cues to feed. Shared influences also affected 'satiety responsiveness', although to a slightly smaller extent (12%), in keeping with the estimate observed by Carnell et al (2008) in the full TEDS sample at age 11 (21%).

Shared environmental factors that potentially play a role in shaping 'food responsiveness' and 'satiety responsiveness' in early life may include 'programming' of appetite through over-nourishment of the twins in utero (excessive maternal weight gain or gestational diabetes), and feeding practices in early postnatal life (e.g. bottle-feeding versus breast-feeding). Experimental research with animals has shown that a maternal diet high in fat and sugar during pregnancy or lactation leads to off-spring that are hyperphagic and have a preference for energy-dense foods (traits indicative of high responsiveness to food cues and low satiety sensitivity) (Bayol et al., 2007; Samuelsson et al., 2008; Schmidt et al., 2001). In each case the hypothesized causal model is epigenetic alteration to the hypothalamic circuits controlling appetite (hypothalamic leptin resistance) which start to develop in utero and continue to mature during early post-natal life (Gluckman & Hanson, 2008; Grattan, 2008).

In humans, an association has sometimes been observed between formula-feeding and accelerated growth in infancy (Owen et al., 2005; Harder et al., 2005; Gluckman & Hanson, 2008; Gillman et al., 2001) which has been hypothesized to result from three potential appetitive pathways: formula-milk tends to be more energy-dense than breast-milk and considerably higher in protein content thus presenting an opportunity for 'over-nourishment'(Heinig et al., 1993); infants may be better able to control their energy intake

in line with their internal satiety cues when breast-fed than when bottle-fed, especially if encouraged to finish the bottle after they are full (Li et al., 2008); breast-milk contains chemical substances that may aid in regulating satiety in the infant, such as leptin (Savino & Liguori, 2008). Two studies have provided some evidence that over-nutrition through formula feeding does lead to excess weight in human infants – babies randomised to receive a lower protein formula milk had significantly lower weight-for-length scores at 24 months compared with those randomised to receive higher formula (Koletzko et al., 2009), and Singhal and colleagues (2002) found that preterm infants randomised to receive a nutrient-enriched preterm formula had a significantly higher leptin to fat mass ratio at adolescence compared to infants randomised to receive standard formula or banked breast-milk, but with breast-fed babies showing the lowest ratio, implicating appetitive programming effects of formula milk in early life. Interactions between feeding method and the heritability of appetite are discussed in more detail in section 8.5.2.2 below. Presently, little research has been carried out on the early post-natal environmental influences on the development of appetite in human infants, but these findings indicate that an investigation of the shared environmental factors that may be involved may be most fruitfully directed at 'food responsiveness' and satiety responsiveness'.

8.5.2. Summary of subgroup findings

8.5.2.1. Sex differences

'Enjoyment of food' was the only feeding behaviour that appeared to have any substantial sex differences in the ratio of genetic and environmental effects, with confidence intervals that did not overlap. For males heritability was similar to the full sample estimate at 81%, but for females it was somewhat lower (62%); the difference observed was at the cost of the shared environment effect which was negligible for the males (0%), yet modest for the females (22%). These findings are strikingly similar to those of Carnell et al (2008) who reported that the best-fitting model for 'enjoyment of food' permitted quantitative sex-differences in the 11-year old children; their heritability estimates were also very similar to these and the differences were in the same direction (males, 78%; females, 70%), suggesting that there may be persisting sex-differences in the genetic influence on this trait over the developmental period. It is not clear why heritability estimates would be lower

for girls and aspects of the shared environment play a more important role – it may be that parents are more likely to cajole female infants during the feeding interactions than male infants – but replication of this finding using behavioural measures would help to confirm its legitimacy.

8.5.2.2. Feeding method differences

The relative influences of genes and the environment appeared to differ somewhat with feeding method for 'food responsiveness', 'slowness in eating' and 'satiety sensitivity'. A very small gene-environment interaction effect was detected for 'food responsiveness' in that there was a small difference between the two feeding methods in the size of the non-shared environment effect which was slightly (but significantly) smaller for breast-fed infants (0.08) than for bottle-fed infants (0.13), which could indicate that greater differences exist between twins who are bottle-fed due to more variation in aspects of actual feeding and milk; it is not the case that measurement of food responsiveness in bottle-feeding infants is more error prone than measurement of this trait in breast-feeding infants because the Cronbach's alphas were virtually the same and the bottle-feeding alpha was slightly higher (0.78 and 0.80). In addition, heritability was slightly lower for this trait for breast-fed infants (55% versus 66%) and conversely the shared environment effect was a little higher for breast-fed infants than bottle-fed infants (37% versus 20%) with estimates outside the 95% confidence interval for the other group. It may be the case that the genetic propensity to respond to cues to feed is attenuated in the breast-feeding situation by the limited availability of milk – breast-fed infants are therefore less likely to be overfed and the restricted milk supply is a factor shared in common by both twins. On the other hand, bottle-fed infants may be given more opportunity to respond to cues to feed in-line with their natural disposition due to easier access to milk that does not require time for production, which may serve to reinforce the expression of this trait.

The genetic effect for 'slowness in eating' was significantly lower for breast-fed (64%) than bottle-fed (83%) infants, and this difference was also due to a modest effect of the shared environment for infants who were both breast-fed (24%), while no such effect was present for bottle-fed babies (0%). This makes empirical sense given that feeding speed in breast-

fed infants is limited by the flow of milk from the mother, and this factor should influence both of the twins equally, whereas bottle-fed infants may suck as quickly or as slowly as they please, in line with their own genetic propensity. It may indicate that bottle-feeding is more of a problem for infants with avid appetites who will empty the bottle very quickly and potentially override their internal cues of satiety in the process, while breast-feeding can provide a natural limit to the amount of milk the infant is able to consume in a given period of time.

A similar finding was observed for 'satiety responsiveness' in that the genetic influence on this appetitive trait was somewhat smaller for breast-fed infants than for bottle-fed infants (62% versus 78%), and in the same way there was a sizeable influence of the shared environment for breast-feeders (25%) but not for bottle-feeders (6%) with estimates being outside the 95% confidence intervals of the other group for in each case. The same explanation probably holds for this finding as well – the amount of milk that an infant is able to consume when breast-fed may in part reflect the quantity of milk the mother is able to produce as well as the infant's own satiety set-point, with this factor acting to increase similarity between the breast-fed twins; on the other hand, bottle-fed twins may be better placed to consume as much milk as they would like in order to feel satiated, so greater differences are permitted between bottle-fed twins than between breast-fed twins that reflect their different genetic dispositions – this may be observed in the twin correlations for this trait that are significantly lower for bottle-fed dizygotics than for breast-fed dizygotics. In addition to the behavioural explanation there may be biological aspects of breast-milk that influence satiety sensitivity – breast-milk contains leptin but formula milk does not (O'Connor et al., 2003; Resto et al., 2001), breast-fed infants have higher blood leptin concentrations than formula-fed babies (Savino et al., 2005; Savino & Liguori, 2008), and milk leptin levels are associated with infant serum leptin concentration (Savino & Liguori, 2008) suggesting that breast-milk leptin plays a role in the regulation of satiety sensitivity in milk-feeding infants. This factor that the two twins share in common would act to increase the shared environment effect.

Collectively these findings are slightly curious given that breast-fed infants on average were scored as more food responsive, as faster feeders and as less satiety sensitive than bottle-fed infants. However, as mentioned in Chapter 6 it may be the case that the infants

with poorer appetites were more likely to be bottle-fed, and the infants who demonstrated good appetites were more likely to be breast-fed for longer. Nevertheless, these findings require further investigation and replication of feeding method differences in a different sample of infants would add weight to these findings.

8.5.3. Strengths and weaknesses

This study is one of the first to assess the heritability of appetitive traits in the very earliest period of life, during the milk-feeding phase. However, a few limitations must be acknowledged. The BEBQ was completed by the same parent (usually the mother) for both twins. Using the same rater to assess each twin in the pair may introduce rater response biases (such as idiosyncratic response styles) that are shared across co-twins, which tends to inflate the shared environment effect. It would be useful to model the appetite traits using information from more than one rater (e.g. mother and father) in a multivariate model so that the genetic and environmental influences on these traits may be assessed after correlated rater bias has been partitioned out.

The sensitivity analyses suggested that heritability estimates may have been slightly higher following exclusion of problem-feeders but this would have resulted in an unacceptable loss of information and compromised the reliability of the parameter estimates. The size of the differences were small, suggesting that this would not have changed the findings greatly. There were virtually no differences in heritability estimates when calculated using twins whose parents' zygosity classification matched the questionnaire classification, and twins whose parents' classification differed (95% confidence intervals overlapped for all scales), suggesting that parental rating biases in relation to zygosity labelling were not present or very limited.

Lastly, 'enjoyment of food' was modelled as a continuous measure rather than a dichotomous variable (although it was not normally distributed), in order to allow comparison with the other eating behaviours, and with multivariate findings in future chapters. Nevertheless, the estimates may not be as reliable as those for the other appetite scales. As expected, heritability was somewhat lower (53%) when estimated

using categorical methods, but this was in keeping with the results for the normally distributed 'slowness in eating' scale for which heritability was also lower (66%) when modelled dichotomously.

8.5.4. Implications for theory, practice and future research

Finding substantial heritability for all of these appetitive characteristics, as well as associations with weight (Chapter 7), provides another piece of evidence to suggest that appetite sits on the causal path from genotype to adiposity. If this is true, appetite and weight should show a common genetic pathway; this can be tested for using a multivariate genetic analysis. The focus of Chapter 10 will be to explore common pathways underlying appetite and weight.

In addition, finding that these traits are heritable in combination with their inter-relatedness (Chapter 6) raises the possibility that the observed associations between them are being driven by shared genetic pathways underlying all of the traits. The next chapter explores whether it is primarily shared genetic factors or common environmental factors that account for the phenotypic associations between the BEBQ scales, using a multivariate genetic design.

CHAPTER 9. STUDY 4: SHARED PATHWAYS UNDERLYING APPETITIVE TRAITS IN INFANCY

9.1. Background

Chapter 8 provided evidence for substantial genetic influence on 'enjoyment of food', 'food responsiveness', 'slowness in eating', and 'satiety responsiveness' in infancy. The PCA analyses in Chapter 6 found that these appetitive traits are distinct phenomena insofar as they form independent components statistically, although they were interrelated – faster feeding and lower satiety sensitivity tended to be observed in infants who were highly food responsive and enjoyed feeding more, although food responsiveness had a smaller relationship with the other appetitive traits. This clustering of eating behaviours has also been observed in older children (e.g. Carnell et al., 2008; Sleddens et al., 2008; Viana et al., 2008; Wardle et al., 2001b). These patterns raise questions about their shared aetiology – are these eating behaviours correlated because they share genes in common that give rise to them all, or because there are certain environmental factors that promote all of these appetitive traits? Finding that traits are phenotypically correlated and that genes play an important role in shaping these traits raises the possibility that it is shared genes driving the observed associations. Some studies with adults have indicated that there may be a common genetic pathway underlying the eating behaviours of the TFEQ (Keskitalo et al., 2008; Neale et al., 2003a), but this has never been explored with children or infants.

It is possible to answer this question using a multivariate analysis of the genetic and environmental influences on the BEBQ scales. In particular, the available methods make it possible to quantify the extent to which common genetic factors or common environmental factors are responsible for driving the observed phenotypic correlations among the appetitive characteristics; secondly, for each trait in the model the total genetic and environmental variation can be partitioned into that which is trait specific (and independent of the other eating behaviours), and that which is common to the other traits (Neale & Maes, 2001; Plomin et al., 2008). A multivariate approach also has an added benefit over univariate analyses of increased power to detect small effects that were not significant in the simpler models, such as those of the shared environment.

9.2 Study aims

This study uses multivariate methods to explore the role of shared genetic and environmental influences in explaining the observed associations between appetitive characteristics during the first three months of life. The following questions will be addressed:

1. Are shared genes or shared environments driving the phenotypic covariation between these appetitive traits?
2. How much of the trait variation is due to common influences and how much to specific influences?

9.3. Methods

9.3.1. Multivariate heritability analyses

All heritability analyses were conducted on BEBQ scale scores that had been residualised for age and sex effects, using the method described in Chapter 8. Scores for 'slowness in eating' and 'satiety responsiveness' were reversed so that all correlations were in the same direction (with a higher score indicating a larger overall appetite) to aid interpretation of the results. 'Appetite size' was not included in the multivariate analyses because it is theorised to represent appetite as a whole, rather than any one specific trait – the purpose of the multivariate analysis was to ascertain whether distinctive styles of feeding, like those demonstrated in children, share a common genetic or environmental pathway, irrespective of their indication of overall appetite size.

9.3.1.1. Twin correlations

Cross-twin cross-trait intraclass correlations were calculated for every pairwise combination of scales to explore the shared heritability of each pair of traits; for every pair

of traits there were two cross-twin, cross-trait correlations – trait 1 in twin 1 correlated with trait 2 in twin 2, and trait 1 in twin 2 correlated with trait 2 in twin 1. These were compared to the phenotypic correlations that were calculated using Pearson's Product Moment correlation coefficients[79], to ascertain if there were indications of genetic influences common to both traits. The twin correlations were performed in SPSS version 15 for Windows.

9.3.1.2. Second order principal components analysis

While Pearson's correlations were used to estimate the pairwise associations, a second order principal components analysis was used to assess the covariation between the four traits collectively. It was useful to assess the extent to which the four components loaded together on to one component because it allowed me to determine the appropriateness of a Common Pathway Model for the data, which uses a factorial approach (the latent factor). The same principal components method that was described in Chapter 6 was used to explore the relationships between the four scales; the mean scale scores (residualised for age and sex effects) were entered in to the analysis instead of the 18 items, and all twins were included to keep the model as similar as possible to the model that would be generated by Mx in the covariance modeling-fitting for the Common Pathway Model.

9.3.1.3. Covariance model-fitting

A multivariate saturated model was run first in order to provide a comparison for the multivariate genetic models. Two multivariate models were run to examine the shared genetic and environmental influences on the appetitive traits, including a Correlated Factors Model (CFM) and a Common Pathway Model (CPM); an Independent Pathway Model was not run because in the three variable case (only three scales were included,

[79] Spearman's rho was also used to check the correlations between 'enjoyment of food' and the other scales because it was not normally distributed but the results were the same so only Pearson's Product Moment correlation coefficients are reported. The correlation coefficients reported here include all of the twins, and were performed on scores residualised for age and sex effects, therefore they differ slightly to the correlation coefficients reported in Chapter 6 which included only one twin from each family drawn at random and unadjusted scores.

discussed in the results section) it is equivalent to a Cholesky Model insofar as it estimates the same number of parameters and therefore provides no novel information.

Standard ACE models were run because there was no evidence of genetic dominance in the univariate analyses presented in Chapter 8. Each full model was compared to the saturated model, and the CPM was also compared to the CFM within which it is nested. More parsimonious sub-models of the CFM and CPM were also tested against the fuller models within which they were nested using two approaches. Firstly, each combination of parameters (e.g. all genetic correlations) were systematically dropped[80] and the goodness-of-fit of competing models assessed, and secondly, non-significant parameters from the full model were dropped and the resulting model was tested against the full model. None of the specific unique environmental influences were dropped from the model as to do so would be to assume that the BEBQ scales could be measured without error.

Selection of the best-fitting model was based upon BIC changes from fuller models. As mentioned in Chapter 4 both the likelihood ratio test and AIC have a similar drawback in that as the sample size increases there is a tendency to prefer the more complex model (i.e. the model with more parameters) (Raftery, 1995). As BIC takes sample size into account as well as parsimony when evaluating model-fit (Mulaik et al., 1989), and has been found to outperform AIC with very large samples and complex models (Markon & Krueger, 2004), this statistic may be more informative when identifying the best-fitting and most parsimonious model from a number of competing models with large numbers of parameters such as is the case with the multivariate analyses.

For the best-fitting Common Pathway Model the total genetic influence on each trait was calculated by summing the common and specific genetic variances; the specific genetic variance on each trait was estimated by squaring the specific genetic path estimate on that

[80] E.g. for the CFM all of the shared environment parameters were dropped, or all of the additive genetic parameters were dropped, or both were dropped, or all of the shared environment correlations were dropped, or all of the additive genetic correlations were dropped, or all of the unique environment correlations were dropped; for the CPM all of the specific additive genetic parameters were dropped, or all of the specific shared environment parameters were dropped, or all of the specific additive genetic and shared environment parameters were dropped, or the common additive genetic factor was dropped, or the common shared environment factor was dropped, or the common unique environment factor was dropped.

trait; the common genetic influence on each trait was calculated by multiplying the square of the latent variable path estimate on that trait by the square of the common genetic path estimate on the latent variable; the proportion of the total genetic influence explained by common and specific influences was estimated by calculating the percentage of the total genetic variance explained by specific or common genetic influences (the same methods were used to decompose the total shared and unique environmental effects into trait-specific and common influences). The contribution of the common genetic factor to each pairwise phenotypic correlation (i.e. the bivariate heritability) was calculated by multiplying the path estimates from the latent factor to each of the two traits by the square of the path estimate from the common genetic factor to the latent variable (the same method was used to calculate the contribution of the common environmental factors to each pairwise phenotypic correlation); the genetic and environmental contributions to the phenotypic correlation were also converted to percentages to ease interpretation. Genetic (and environmental) correlations were then derived for each pair of appetitive traits from the bivariate heritability estimates and the total genetic influence on each appetitive trait – the bivariate genetic (or environmental) contribution to the phenotypic correlation was divided by the product of the square roots of the total genetic (or environmental) influence estimates on each trait. The covariance modelling was conducted using Mx Maximum-Likelihood Structural Equation Modelling Software (version 32; Virginia Commonwealth University, Richmond, VA).

9.4. Results

9.4.1 Twin correlations

The cross-twin cross-trait correlations between ‘enjoyment of food’, ‘food responsiveness’, ‘slowness in eating’ and ‘satiety responsiveness’ are shown in Table 9.1, with the phenotypic correlations alongside for comparison. For cross-twin cross-trait associations between ‘enjoyment of food’, ‘slowness in eating’ and ‘satiety responsiveness’, all the MZ correlations were moderate and significant, while the DZ correlations were small (slightly less than half the MZ correlations) but also significantly different from zero. This pattern of

results indicates that for these pairwise combinations of traits, shared genes are contributing to the phenotypic correlations observed, but common shared environmental factors are not. ‘Food responsiveness’ did not give any indication of common genetic or common shared environmental pathways with ‘enjoyment of food’ or ‘slowness in eating’ as the MZ correlations were not significantly different from zero, suggesting that the phenotypic association is largely explained by common unique child environmental effects. However, ‘food responsiveness’ was significantly associated with ‘satiety responsiveness’ in MZs, while the DZ correlation was not significantly different from zero or significantly negative, suggesting that the relationship between these two traits may be partly due to shared genes, although the phenotypic correlation was small. However, on the whole the very small phenotypic correlations between ‘food responsiveness’ and the other traits, as well as different cross-twin cross-trait correlation patterns, suggested that this scale should not be included in the multivariate covariance model-fitting analyses.

Table 9.1. Cross-twin cross-trait intraclass correlations and phenotypic correlations of appetitive traits

BEBQ[a] Scales	Twin[b] and Scale[a]	ICC[c] (95% Confidence Interval)		Phenotypic Correlations[e]
		MZ[d]	DZ[d]	
'enjoyment of food' & 'food responsiveness'	Twin 1 EF * Twin 2 FR	**0.04** (-0.04, 0.11)	**-0.12** (-0.17, -0.07)	**0.08**
	Twin 2 EF * Twin 1 FR	**0.05** (-0.03, 0.12)	**-0.11** (-0.16, -0.06)	
'enjoyment of food' & 'slowness in eating'	Twin 1 EF * Twin 2 SE	**0.36** (0.29, 0.42)	**0.10** (0.05, 0.15)	**0.41**
	Twin 2 EF * Twin 1 SE	**0.41** (0.35, 0.47)	**0.15** (0.10, 0.20)	
'enjoyment of food' & 'satiety responsiveness'	Twin 1 EF * Twin 2 SR	**0.41** (0.34, 0.47)	**0.16** (0.11, 0.21)	**0.49**
	Twin 2 EF * Twin 1 SR	**0.39** (0.33, 0.45)	**0.19** (0.14, 0.24)	
'food responsiveness' & 'slowness in eating'	Twin 1 FR * Twin 2 SE	**0.03** (-0.05, 0.10)	**-0.13** (-0.18, -0.08)	**0.10**
	Twin 2 FR * Twin 1 SE	**0.04** (-0.04, 0.11)	**-0.16** (-0.21, -0.11)	
'food responsiveness' & 'satiety responsiveness'	Twin 1 FR * Twin 2 SR	**0.13** (0.06, 0.20)	**-0.04** (-0.09, 0.01)	**0.21**
	Twin 2 FR * Twin 1 SR	**0.14** (0.07, 0.21)	**-0.10** (-0.15, -0.05)	
'slowness in eating' & 'satiety responsiveness'	Twin 1 SE * Twin 2 SR	**0.36** (0.30, 0.42)	**0.12** (0.07, 0.17)	**0.44**
	Twin 2 SE * Twin 1 SR	**0.34** (0.27, 0.40)	**0.07** (0.02, 0.12)	

[a] BEBQ, Baby Eating Behaviour Questionnaire; EF, enjoyment of food; FR, food responsiveness; SE, slowness in eating; SR, satiety responsiveness.
[b] Randomly allocated twin (1 or 2) and scale used in the cross-twin cross-trait correlation.
[c] ICC, intraclass correlation.
[d] MZs, *n*=708-718 pairs; DZs, *n*=1565-1585 pairs.
[e] Pearson's product-moment correlation coefficients; *n*=4677-4727.

9.4.2 Second order principal component analysis

The second order principal component analysis supported the findings from the pairwise correlations – 'enjoyment of food', 'slowness in eating' and 'satiety responsiveness' all loaded on to a common factor with loadings of 0.78, 0.75 and 0.82 respectively. On the other hand, 'food responsiveness' did not load above 0.4 (0.33) suggesting that a much smaller amount of variance in this scale was explained by the covariance with the other three scales. The total amount of variance in the four scales together explained by the common factor was 49%. The PCA was run again without 'food responsiveness' and the loadings of the three scales were very similar ('enjoyment of food', 0.80; 'slowness in eating', 0.77; 'satiety responsiveness', 0.82), but the common factor explained a much greater amount of the variance in the three scales collectively (63%). These analyses, together with the twin correlations, suggested that 'food responsiveness' should not be included in a common pathway model.

9.4.3 Covariance model-fitting

9.4.3.1. Selection of the best-fitting multivariate model

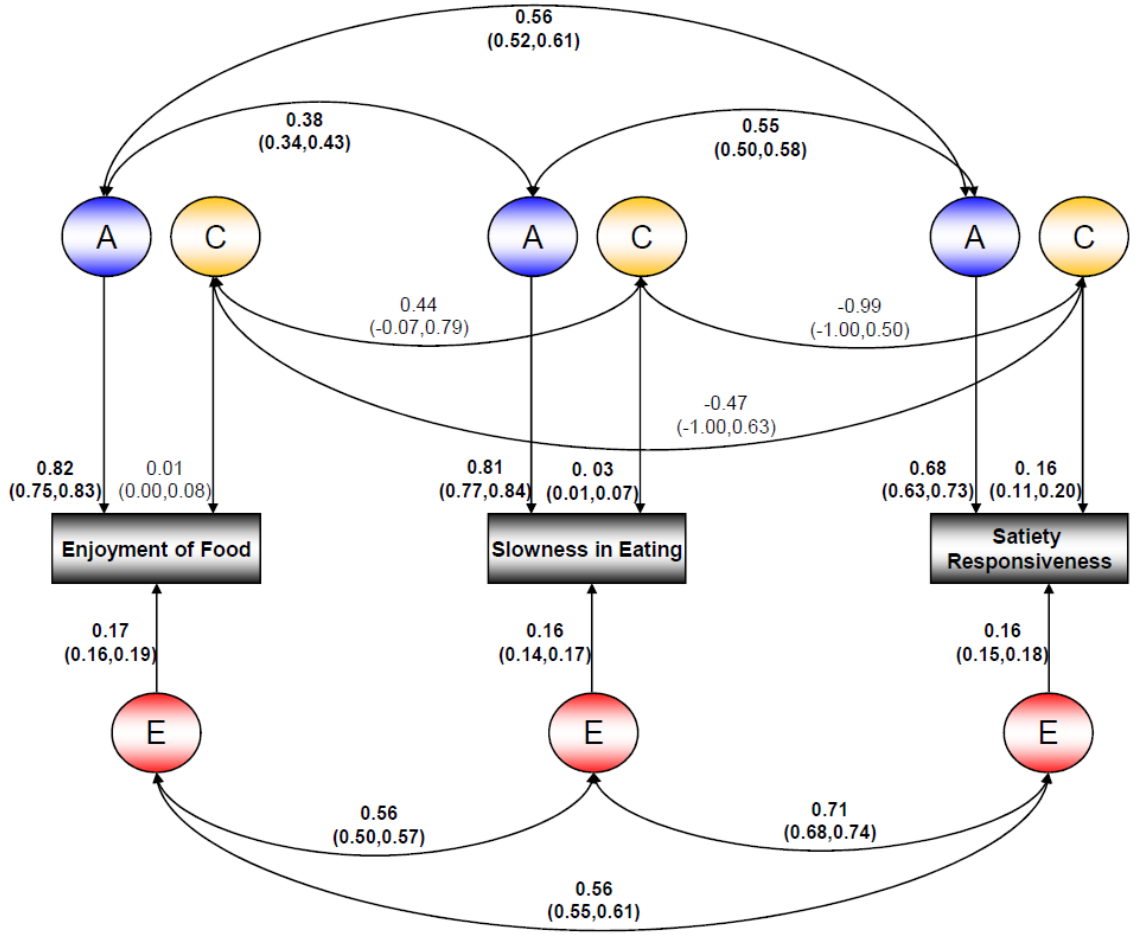
The multivariate models included 'enjoyment of food', 'slowness in eating' and 'satiety responsiveness'. The goodness-of-fit statistics for the many models that were tested are shown in Appendix 6.1. The BIC difference between the full CFM and the full CPM was not large enough to confidently select one of the models over the other (2.696), although the principle of parsimony would suggest that the CPM be preferred (the parameter estimates for the full CFM are shown in Figure 9.1 and the parameter estimates for the full CPM are shown in Figure 9.2). The first approach to identify sub-models by systematically dropping parameters indicated that there were no common shared environmental influences – it was possible to drop all of the shared environment correlations from the CFM, and the common shared environment factor from the CPM. The resulting sub-models both indicated that there was also no specific shared environment effect for 'enjoyment of food' as this was estimated as zero in each case, and the shared environment effect for 'slowness in eating' was also very small in each case (5%) and not significantly different from zero, suggesting

that these two parameters could also be dropped from both models. Dropping these parameters resulted in the best-fitting models for the CFM and CPM and both were equivalent; the best-fitting CFM included all of the additive genetic parameters, all of the unique environment parameters, and the shared environment parameter only for SR, as well as all of the additive genetic correlations, all of the unique environment correlations, but no shared environment correlations; the best-fitting CPM model included all of the specific additive genetic and unique environment parameters, and only the specific shared environment parameter for SR, and included the common additive genetic and unique environment parameters, but not the common shared environment parameter. The BIC difference between these two models was too small to favour one model over the other with the CFM having a slightly lower value by 1.382, which provides only 'weak' evidence for the CFM over the CPM according to the guidelines (Raftery, 1995). However, following the principle of parsimony the CPM offers a less complex multivariate representation of the shared pathways underlying the eating behaviours and this model was therefore preferred.

The parameter estimates for the preferred sub-model of the CPM are shown in Figure 9.3[81] and the estimates derived from the path diagram (total, common and specific genetic and environmental influences, phenotypic covariation, bivariate estimates of heritability, shared environment and unique environment effects, genetic and environmental correlations) are presented in Tables 9.2 and 9.3. As can be seen, the parameter estimates of the full CPM (Figure 9.2) and those of the preferred sub-model of the CPM (Figure 9.3) are virtually the same, indicating that dropping the non-significant parameters did not alter the model. Only the findings from the best-fitting sub-model are therefore discussed in the next section (9.4.3.2).

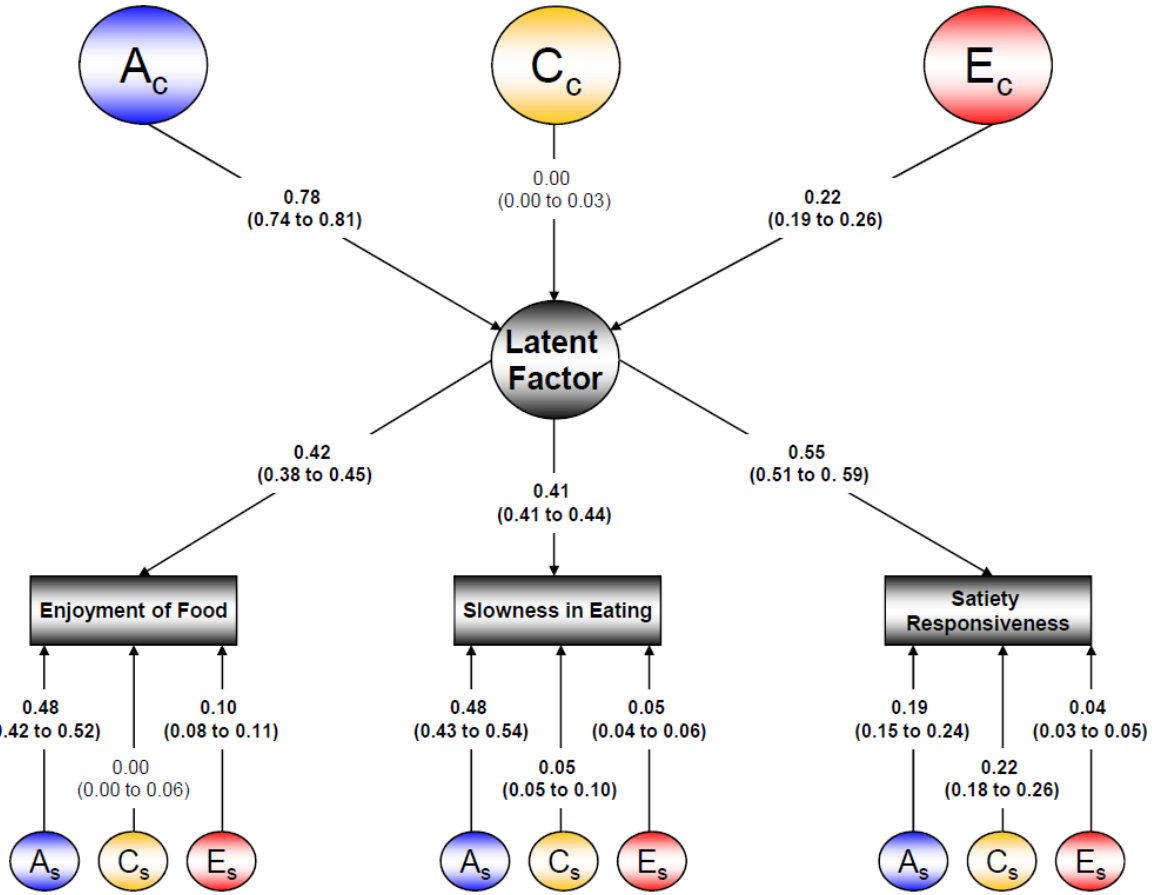
[81] The path diagram shows the standardised variance components (the squares of the standardised path estimates) rather than the standardised path estimates as this information shows the amount of variance explained in the BEBQ scales by common and specific influences, providing more useful information.

Figure 9.1. Full ACE Correlated Factors Model showing the univariate genetic and environmental influences on 'enjoyment of food', 'slowness in eating' and 'satiety responsiveness'



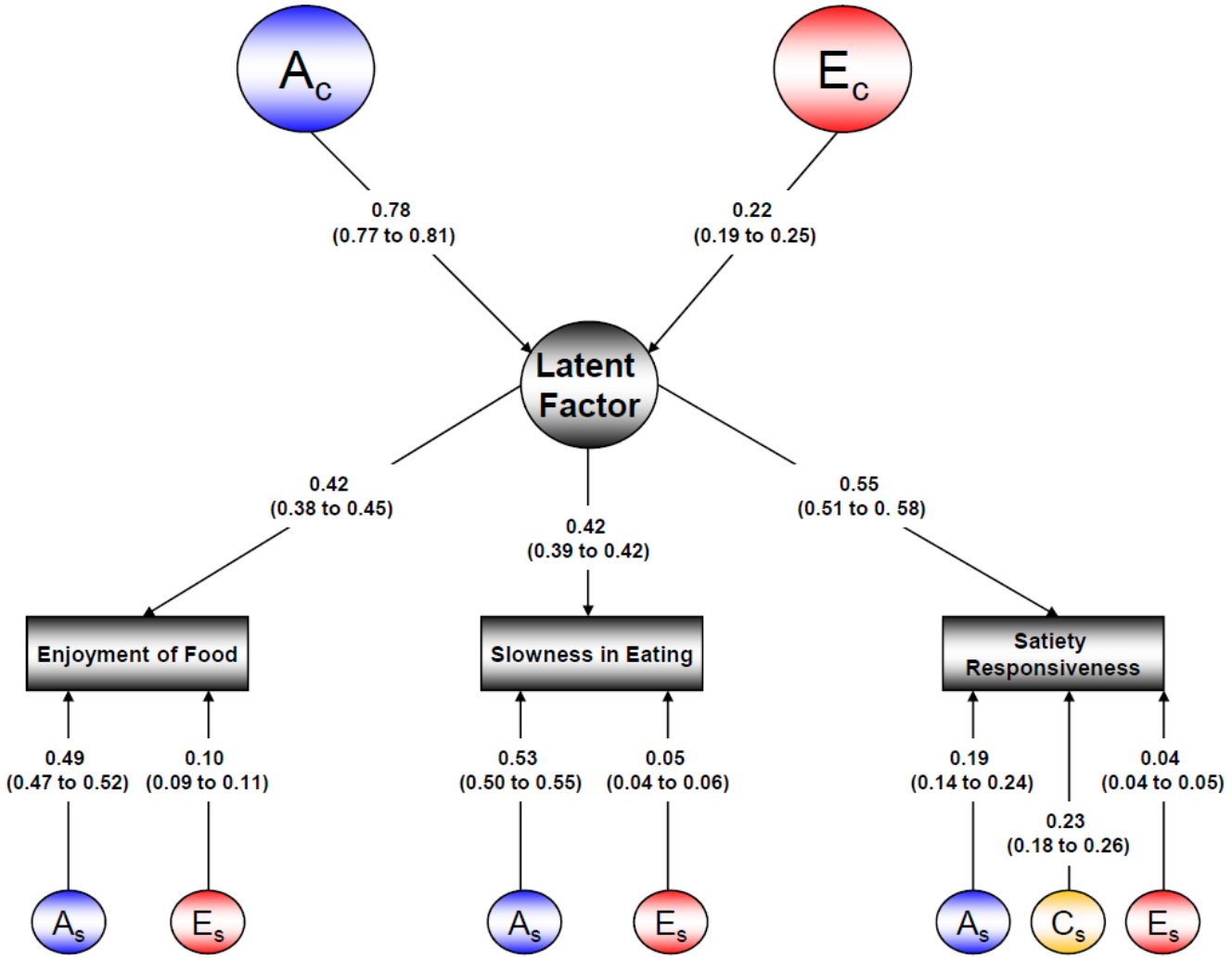
Path diagram showing the genetic and environmental influences on 'enjoyment of food', 'slowness in eating' and 'satiety responsiveness' for one child using a Correlated Factors Model. The rectangular boxes represent the measured phenotype (BEBQ scale). The circles indicate latent influences on the measured phenotype which include additive genetic effects (A), shared environmental effects (C) and unique environmental effects and error of measurement (E). The straight single-headed arrows show the causal paths, and the squared path coefficients on each causal path indicate the total variance explained in each eating behaviour by A, C and E. The curved double-headed arrows show the genetic, shared environment and unique environment correlations between the traits which can range between 0 and 1. Significant parameter estimates for which the 95% confidence interval did not include zero are bolded.

Figure 9.2. Full ACE Common Pathway Model showing the multivariate genetic and environmental influences on 'enjoyment of food', 'slowness in eating' and 'satiety responsiveness'



Path diagram showing the genetic and environmental influences on 'enjoyment of food', 'slowness in eating' and 'satiety responsiveness' for one child using a Common Pathway Model. The rectangular boxes represent the measured phenotypes (BEBQ scales). The circles indicate latent influences on each BEBQ scale; these include common influences that contribute to covariation between the traits (common additive genetic effects (Ac), common shared environmental effects (Cc) and common unique environmental effects (Ec)) mediated through a latent factor that explains variance in the eating behaviours, as well as influences specific to each trait (specific additive genetic influences (As), specific shared environmental influences (Cs) and specific unique child environmental influences and error of measurement (Es)). The straight single-headed arrows show the causal paths, and the squared path coefficients on each causal path indicate the total variance explained in the latent factor or the measured phenotypes by the latent genetic and environmental influences. Significant parameter estimates for which the 95% confidence interval did not include zero are bolded.

Figure 9.3. Preferred Common Pathway Sub-model showing the multivariate genetic and environmental influences on 'enjoyment of food', 'slowness in eating' and 'satiety responsiveness'



See footnote for Figure 9.2.

9.4.3.2. Multivariate findings from the best-fitting model

Overall, a Common Pathway Model was preferred that included common genetic and unique environmental influences on ‘enjoyment of food’, ‘slowness in eating’ and ‘satiety responsiveness’, as well as specific genetic and unique environmental influences on all of the traits, and specific shared environmental influences only on ‘satiety responsiveness’ (Figure 9.3). This suggests that during infancy there are a set of common genetic influences and common unique environmental influences that all contribute significantly to the covariation between the three appetitive characteristics; on the other hand, there are no common shared environmental factors that contribute to the observed associations between the three traits. It was also clear that there were important specific influences that contributed to trait distinction as it was not possible to constrain all additive genetic influences (and shared environmental influence for ‘satiety responsiveness’) to zero. In order to aid interpretation of the information from the best-fitting model, the proportion of variance from the total genetic effects and the total environmental effects on each trait are shown in Table 9.2. For each BEBQ scale the total amounts of genetic and environmental variation have been further broken down in to the proportions from specific and common influences. The contribution of the common genetic and common unique environmental factors to pairwise phenotypic correlations have also been summarized as bivariate heritability and bivariate environmental influences, and genetic and environmental correlations have been calculated using these and are presented in Table 9.3.

A number of key observations stand out from the findings. Firstly, a common genetic factor was most instrumental in driving the observed covariation between these three characteristics as this common factor explained a much greater proportion of the variance in the latent factor than common unique environmental influences (78% versus 22%). This is demonstrated further by the genetic and environmental contributions to the pairwise correlations between the three BEBQ scales (Table 9.3) – the majority of each pariwise correlation was explained by common genetic influences, rather than unique environmental factors that were common to each pair of traits.

Secondly, the latent factor explained a slightly (but significantly) greater proportion of the variance in ‘satiety responsiveness’ (55%) than the other two appetitive traits (42% in each case) making this the characteristic that had most influences in common with the other traits, in keeping with the pairwise phenotypic correlations (Table 9.1). The loadings of the latent factor on to the scales indicated that while common influences explained the majority of the variance in ‘satiety responsiveness’, unshared genetic and environmental influences that were specific only to ‘enjoyment of food’ or ‘slowness in eating’ were more important for explaining variance in these traits.

Thirdly, the univariate estimates reported in Chapter 8 were more or less recovered (Table 9.2). The total heritability of ‘enjoyment of food’ and ‘slowness in eating’ remained very high (81% and 85% respectively), and the total genetic influence on ‘satiety responsiveness’ was still modest to high (63%), although a slightly greater influence of the shared environment was detected for this trait here than in the univariate analyses reported in Chapter 8; no such effect was detected for the other two traits, in keeping with the estimates obtained from the univariate models in Chapter 8.

Lastly, a slightly greater proportion of the total genetic influence for ‘enjoyment of food’ and ‘slowness in eating’ came from genes specific to those traits, while genetic influences in common with the other eating behaviours are more important for ‘satiety responsiveness’; this is shown by the relative magnitudes of the genetic correlations that are slightly higher between ‘satiety responsiveness’ and the other two eating behaviours than that between ‘enjoyment of food’ and ‘slowness in eating’ for which specific influences are slightly more important (Table 9.3).

Table 9.2. Percent of variance explained in 'enjoyment of food', 'slowness in eating' and 'satiety responsiveness' by common and specific genetic and environmental influences from the preferred Common Pathway sub-model

BEBQ Scales[a]	Genetic Influences			Shared Environmental Influences		Unique Environmental Influences		
	Total[b]	Common (%)	Specific (%)	Total[b]	Specific (%)	Total[b]	Common (%)	Specific (%)
EF	0.82	0.32 (40)	0.49 (60)	0.00	0.00 (0)	0.19	0.09 (47)	0.10 (51)
SE	0.85	0.32 (38)	0.53 (62)	0.00	0.00 (0)	0.14	0.09 (64)	0.05 (36)
SR	0.62	0.43 (69)	0.19 (31)	0.23	0.23 (100)	0.16	0.12 (75)	0.04 (25)

[a] Abbreviations: BEBQ, Baby Eating Behaviour Questionnaire; EF, 'enjoyment of food'; SE, 'slowness in eating'; SR, 'satiety responsiveness'.
[b] Total variance of each trait may not add up to 1.0 due to rounding.

Table 9.3. Pairwise phenotypic correlations, bivariate parameter estimates and aetiological correlations for the preferred Common Pathway sub-model for 'enjoyment of food', 'slowness in eating' and 'satiety responsiveness'

BEBQ Scales[a]	Phenotypic correlation	Variance components for bivariate A and E[b] (as % of phenotypic correlation[c])		Aetiological correlations[d]	
		A	E	r_g	r_e
EF*SE	0.42	0.32 (76)	0.09 (24)	0.39	0.56
EF*SR	0.48	0.37 (77)	0.11 (23)	0.53	0.61
SE*SR	0.48	0.37 (77)	0.11 (23)	0.51	0.70

[a] Abbreviations: BEBQ, Baby Eating Behaviour Questionnaire; EF, 'enjoyment of food'; SE, 'slowness in eating'; SR, 'satiety responsiveness'.
[b] Proportion of variance in the phenotypic correlation that is explained by common additive genetic influences and common non-shared environmental influences. The sum of the bivariate components equals the phenotypic correlation.
[c] The proportions of variance in the phenotypic correlations (accounted for by additive genetic effects, shared environment effects and non-shared environment effects) converted to percentages for ease of interpretation.
[d] r_g, genetic correlation; r_e, unique environmental correlation. A genetic or unique environmental correlation is significant if the 95% confidence interval does not include zero; all genetic and unique environmental correlations in the model were statistically significant.

9.5. Discussion

9.5.1. Summary of findings

As far as I am aware this is the first investigation into the shared influences underlying appetitive traits during infancy. In particular, this study provided novel information to explore how common influences are organized, and to quantify the relative importance of shared genetic and environmental factors in driving the observed phenotypic associations. Two questions were addressed by this study and the results are discussed within the context of each, below.

9.5.1.1. Do shared genes or shared environments drive the phenotypic covariation between these appetitive traits?

Both the cross-twin cross-trait correlations and the covariance modeling suggested that covariation between the appetitive traits was caused by genetic influences and common influences of the unique child environment, but no evidence for common shared environmental influences being involved. Shared genes appeared to play a much more important role than environmental influences in generating the phenotypic associations observed between the traits, as 78% of the variance in the latent factor was explained by common genetic influences – that is, infants who are less sensitive to internal cues of satiety, tend also to feed at a faster pace and enjoy milk to a greater extent because these three traits spring from the same underlying genetic influences. Moreover, the pairwise genetic correlations were moderate to high (0.39 to 0.53) indicating that a substantial proportion (up to half) of the genes that influence any one of these appetitive traits also influences another. Together, these findings suggest that although 'enjoyment of food', 'slowness in eating' and 'satiety responsiveness' only covary moderately, the largest part of this covariation is due to common genes – i.e. they tap some general appetite pathway that is primarily regulated by genes – and that up to half of the genetic effects on each trait are the same.

Shared pathways underlying the TFEQ traits in adults have been explored using similar modeling techniques (Neale et al., 2003a; Keskitalo et al., 2008). Neale and colleagues (2003a) found that 'disinhibition' and 'hunger' loaded significantly on to a latent factor (with a large pairwise phenotypic correlation of 0.79), the heritability of which was about 40% (and the genetic correlation was 0.39) while unique environmental influences explained the majority of the variance, and shared environmental factors were estimated as zero. Similarly, another study found modest to large genetic correlations between 'emotional eating', 'cognitive restraint' and 'uncontrolled eating' (0.42 to 0.75) indicating considerable genetic overlap (Keskitalo et al., 2008), but no shared environmental influences contributing to covariation in a modified version of the TFEQ. So, in adulthood there also appears to be an underlying appetitive trait that is in part driven by genetic factors, although environmental factors are slightly more important.

Quantitative genetic research has shown consistently that common genes underlie individual differences across a number of behavioural traits within any given domain. Just a few of these examples include: cognitive abilities such as spatial, memory, verbal and information processing abilities (Carroll, 1993; Jensen, 1998; Luciano et al., 2003; Petrill, 1997; Plomin & DeFries, 1998), and mathematics, language and reading (Plomin & Kovas, 2005); personality traits and psychopathology (Fanous et al., 2002; Hettema et al., 2004; Hettema et al., 2006; Jardine et al., 1984); different psychiatric illnesses (Kendler et al., 1992b; Cardno & Gottesman, 2000; Kendler et al., 1992a); substance use and abuse (Hettema et al., 1999; Kendler et al., 2003); autistic and ADHD behaviours (Ronald et al., 2008); and different cardiovascular disorders such as hypertension, Raynaud's phenomenon and migraine (Williams et al., 2004), as well as hypertension, diabetes and obesity (Carmelli et al., 1994). The phenomenon of common genes underlying a diverse array of cognitive abilities led researchers from this field to put forward the 'generalist genes' hypothesis for cognition (Kovas & Plomin, 2006; Plomin et al., 2007) which proposes that the same genes affect many cognitive characteristics by being expressed throughout the brain, rather than being expressed locally in a specific region (Plomin et al., 2007). As demonstrated by the evidence for genetic commonality across other psychological, behavioural and physiological domains, this hypothesis can be extended to other broad areas such as appetite, as suggested by the findings here.

The concept of pleiotropy supports the 'generalist genes' theory. Pleiotropy refers to the profuse or multiple effects of genes across various biological levels and is a well-established phenomenon that has been widely observed for hundreds of genes – examples include genes being expressed in more than one tissue type, or genes that regulate several intracellular signal transduction pathways (Dudley et al., 2005). An example is the gene responsible for brain-derived neurotrophic factor (BDNF) (Kovas & Plomin, 2006; Plomin et al., 2007), a protein that belongs to the nerve growth family and plays an important role in facilitating growth and differentiation of new neurons, as well as sustaining existing neurons across the brain (Acheson et al., 1995; Huang & Reichardt, 2001). It is expressed in the brain (particularly in the cerebellum, cortex, caudate nucleaus, amygdala, thalamus, corpus callosum, and dorsal root ganglia), and various other parts of the body including the retina, prostate, kidneys, and central nervous system (Su et al., 2004). A variant in this gene has been shown to relate to adiposity (Speliotes et al., 2010), and in mice, deletion of BDNF in the ventromedial and dorsomedial hypothalamus results in hyperphagia and obesity (Unger et al., 2007), raising the possibility that this particular gene has more general effects that could influence a wide range of appetitive mechanisms. Another example of pleitropy is that individuals with a major mutation in the leptin receptor gene show marked disruption to both homeostatic and hedonic appetite regulation processes in the brain (Farooqi et al., 2007a).

It is also of interest that *FTO* is ubiquitously expressed in both foetal and adult tissue, including adipose tissue, pancreatic islets, hepatic cells and various areas of the brain including the cerebellum, hippocampus and hypothalamus, although it is most widely expressed in the hypothalamus (Stratigopoulos et al., 2008; Gerken et al., 2007; Frayling et al., 2007; Fredriksson et al., 2008; Lein et al., 2007; Olszewski et al., 2009). The systemic occurrence of *FTO* raises the possibility that it can influence more than one phenotype by action at a variety of sites or levels involved in appetite regulation. There is behavioural evidence for this. *FTO* has been associated with a range of appetitive traits in children, as discussed in Chapter 8, including 'satiety responsiveness' measured using the CEBQ (Wardle et al., 2008b), 'eating in the absence of hunger' (Wardle et al., 2009), and energy intake (Cecil et al., 2008; Timpson et al., 2008). Variants in or near MC4R have also shown influence on a range of appetitive traits such as increased snacking, total energy and macronutrient intake in children (Stutzmann et al., 2009). However, few genes

have been explored so far in relation to appetite control, and none have been looked at during infancy.

The size of the genetic effect on the latent appetite factor in infants (78%) suggests that multiple genetic variants are likely to be involved in the regulation of appetite more generally. In addition to *FTO* and MC4R a number of other genetic variants have been identified that relate to a range of appetitive characteristics: a missense mutation in the neuromedin-β gene has been associated with higher scores for TFEQ 'disinhibition' and 'hunger' (Bouchard et al., 2004b); a polymorphism in the serotonin receptor gene has found to relate to lower energy intake (Aubert et al., 2000; Herbeth et al., 2005); higher daily energy intake and higher fat intake relates to a polymorphism in the apolipoprotein A-II gene promotor (Corella et al., 2007); variants in the glucose transporter type 2 gene and the human Agouti-related protein gene have been related to differences in macronutrient intakes (Eny et al., 2008; Loos et al., 2005b); bitter taste perception appears to be influenced by variants of the TAS2R38 gene (Drayna et al., 2003; Kim et al., 2003) which also relate to food preferences (Mennella et al., 2005); polymorphisms in TaqIA, that regulates the dopamine D2 receptor gene, influences food responsiveness and food reward (Epstein et al., 2007; Stice et al., 2008; Felsted et al., 2010); and variants in genes that code for appetite hormones or neurotransmitters have also been found to relate to a range of appetitive characteristics (den Hoed et al., 2008; Ghoussaini et al., 2007; Gueorguiev et al., 2009). Polygenicity (many genes influencing any complex trait) has been hypothesized to amplify the pleiotropic effects of generalist genes because each gene has a very small effect so there are likely to be very many physiological or neural mechanisms that all contribute a small amount to the final measured phenotype (Kovas & Plomin, 2006; Plomin et al., 2007).

There was a small but significant role for common influences of the unique environment in the relationship between the three BEBQ scales, as it was not possible to explain the covariation using only a common genetic factor; 22% of the covariation between 'enjoyment of food', 'slowness in eating' and 'satiety responsiveness' (the latent factor) was explained by a common unique child environment factor, which does not include measurement error, but rather captures systematic covariation across traits for each child that comes from environmental influences that are not shared with their co-twin.

Conceivably this could include differences between the children in feeding method such as bottle-feeding one child and breast-feeding the other, or unshared aspects of the uterine environment such as placental factors that differentially affect nutrient transfer (such as chorionicity, placental fusion and central vs. peripheral insertion of the umbilical cord), that can create differences even between MZ twins sharing a placenta (Loos et al., 2005a; van Baal & Boomsma, 1998), and may act to differentially influence the development of the hypothalamus. Another possible example would be substantial differences between twin pairs in birth weights that would result in discordant appetites (on the whole) for babies whose appetites may be up- or down-regulated in an attempt to grow faster or slower than their co-twin during the process of normalization.

9.5.1.2. How much of the trait variation is due to common influences, and how much to specific influences?

Also of interest was the finding that about half of the variance in these appetitive traits was independent of the other traits, suggesting that there are also aspects of each characteristic that are independently controlled by genes unique to that dimension of appetite, or by environmental factors that are unrelated to the other scales. It may be the case that the independent variance observed reflects distinct appetitive mechanisms, or alternatively the different scales may tap other behavioural or psychological traits unrelated to appetite that are influenced by both genetic and environmental influences, such as personality. For example, 'enjoyment of food' may also in part tap an infant's temperament insofar as some children tend to be contented across many domains, of which feeding is one; in addition, an infant's satiety sensitivity may also reflect their level of environmental awareness and interaction – those babies who are less easily distracted by extraneous factors during feeding may be more attentive to internal cues of satiety; similarly, the speed with which an infant finishes a bottle could potentially also provide an indication of that child's impulsivity level. In support of this, Haycraft and colleagues (unpublished data) found that greater 'enjoyment of food' measured using the CEBQ was related to less shyness and greater sociability, while greater 'satiety responsiveness' was associated with greater shyness and greater emotionality measured using the EAS Temperament Survey for Children (Buss & Plomin, 1984). It is possible to test this

hypothesis using a multivariate genetic model including appetite and personality measures.

Nevertheless, these results are to some extent supported by neurobiological research which has shown that the homeostatic control of hunger and satiety are governed by different populations of neurons within the hypothalamus, as described in Chapter 2 (Cone et al., 2001). At the same time, different areas of the brain control hedonic aspects of appetite such as reward, which is regulated by dopamine pathways, opioid systems and endocannabinoids (Zheng & Berthoud, 2008). To further complicate matters there are signals at both the level of the brain ('top-down' regulation of appetite), and 'bottom-up' regulation of hunger and satiety via hormones released by the gastro-intestinal tract (Druce & Bloom, 2006), and cross-talk between homeostatic and hedonic brain systems (Lutter & Nestler, 2009). It is likely that there are highly specific genes governing these processes as well as the 'generalised genes' that account for covariation between them – for example, FTO appears to primarily regulate satiety sensitivity through action in the arcuate nucleus of the hypothalamus, but it may also influence leptin levels which can in turn modulate both satiety sensitivity and reward (Figlewicz, 2003). More work is needed to understand which neurobiological and endocrinological mechanisms control the different appetitive traits captured in the BEBQ, and how genes influence neural and physiological processes both specifically and generally. This is discussed in more detail in Chapter 12.

9.5.2. Implications for theory, practice and future research

These findings have implications for molecular genetic research. The genetic correlations between the traits were moderate, indicating that if a gene were found to influence satiety sensitivity then it is likely to influence level of subjective reward experienced when feeding, as well as feeding speed to some extent. This implies that the search for 'generalist genes' relating to appetite will benefit from exploring aspects of the traits that are in common with one another rather than specific aspects of the traits (Plomin & Spinath, 2004) – a possible avenue may be to use the component scores created from a second order principal component analysis of the final scales and relate these to candidate genetic variants. At the same time, the correlations were far from complete (much less than 1.0), making it

clear that there is a great deal of genetic heterogeneity in the dimensions of appetite, with at least half of the genes that influence each trait being unique to that particular characteristic. The wider search for genes underlying appetite would therefore benefit from measuring the many dimensions that characterize appetite in order to obtain a full picture.

These findings also highlight that if researchers are to identify aspects of the environment that are playing a role in up-or down-regulating appetite as a whole during early life it will be necessary to take measures of candidate influences for each individual child, not just of the shared rearing environment. Methods for identifying non-shared environmental influences are discussed in more detail in Chapter 12.

A point for consideration is the extent to which elements of these dimensions of appetite truly reflect one underlying pathway (insofar as they form one latent component). It is possible that the different measurement scales tap aspects of all of the appetitive traits, while primarily measuring the target characteristic and thus the component is an artifact of measurement rather than a unified underlying entity. It may also be the case that the appetitive traits spring from independent genetic pathways but affect one another at the phenotypic level, giving the impression that they share genes in common – e.g. faster-feeding infants may be less sensitive to internal cues of satiety simply because the process of rapid feeding results in the infant consuming too much milk before internal satiety mechanisms have had a chance to develop. Methods for deciphering shared genetic pathways are discussed in Chapter 12.

A question that has been raised by the analyses conducted in this chapter is why 'food responsiveness' showed a different relationship with the other appetite scales at this age. It was not very highly correlated with the other eating behaviours (Chapter 6) and did not show cross-covariance with the other traits in the twin correlations. A different picture is observed with children and adults – this trait measured using the CEBQ is modestly correlated with the other traits in children (Carnell & Wardle, 2007; Sleddens et al., 2008; Viana et al., 2008; Wardle et al., 2001b), and this trait bears some resemblance to 'disinhibition' measured using the TFEQ in adults which was shown to share genetic underpinnings with another appetitive trait 'hunger' in adult life (Neale et al., 2003a). It may

be that responsiveness to milk or cues to suckle in infancy are distinct from responsiveness to highly palatable food cues in the later childhood and adult environment. Another possibility is that the genes that influence this trait are more specific, and 'generalised genes' play less of a role for this trait. A recent animal study reported that changes were observed in hypothalamic FTO expression in the mouse brain in response to hunger (in a food deprived state) and satiety (following intake) but not in response to reward cues (upon exposure to highly palatable foods), and neurons that express FTO primarily regulate feeding termination mechanisms (Olszewski et al., 2009). Perhaps 'food responsiveness' during infancy is a trait less influenced by FTO (and other potential common genes) than the other characteristics. More insight into this scale and its relationship with the other appetitive characteristics will be gained from the behavioural validation work that is currently underway, and ultimately from exploring its relationship with molecular genetic variants.

9.5.3. Strengths and weaknesses

This was the first study the explore to extent to which underlying genes or common environmental influences drive phenotypic associations between appetitive characteristics during the earliest period of life. Nevertheless, the developmental status of the sample limits generalizations about the genetic architecture of appetite to infancy. It is possible that the organization of appetite is different later in childhood – for example, researchers have shown that common genetic influences underlying cognitive abilities increase over the lifespan with genetic correlations in the order of nearly 1.0 by late adulthood (Petrill, 2002). It will be possible to explore how the organization of appetite changes over the first five years of childhood in Gemini.

CHAPTER 10. STUDY 5: SHARED PATHWAYS UNDERLYING APPETITE AND WEIGHT IN INFANCY

10.1. Background

The overarching aim of this thesis was to provide new evidence to support the behavioural susceptibility model of obesity, by exploring the relationship between appetite and weight during the first three months of life, while infants are still exclusively fed milk. In particular, finding shared genetic pathways underlying appetite and weight this early on in the life cycle would help to identify potential causal processes underlying rapid weight gain, and provide evidence for this theoretical model.

The preceding chapters have contributed to this evidence base so far by showing that from the beginning of life there are considerable individual differences in a range of appetitive characteristics akin to those observed in children and adults (Chapter 6), that these traits are associated with weight at 3 months (Chapter 7), and that the characteristics are highly heritable (Chapters 8 and 9) in keeping with the literature on weight (Beardsall et al., 2009; Dubois et al., 2007b; Gielen et al., 2008; Levine et al., 1987; Lunde et al., 2007; Pietilainen et al., 2002; Vlietinck et al., 1989; Whitfield et al., 2001). Collectively, these findings raise the possibility that the genes that influence weight are working through appetitive pathways – that is, the same genes that influence appetite also have an influence on weight. Nevertheless, the phenotypic associations between these traits and weight were only modest, so it is clear that most of the genetic or environmental influences on each are not in common, so a more important but related issue is the extent to which shared genes (or shared environmental factors) are driving the observed associations.

Finding shared genes underlying both appetite and weight during infancy, and finding that shared genes account for a substantial proportion of the observed phenotypic associations (reported in Chapter 7), would contribute considerably to the evidence base that genes are influencing weight in part through appetitive pathways, from the beginning of life. As far as I am aware no-one has ever looked at shared pathways underlying appetitive traits and weight during infancy. This analysis will provide the last piece in the puzzle for the questions that I set out to answer in this thesis.

10.2 Study aims

The overall aim of this chapter is to explore shared genetic and environmental pathways underlying appetite and weight at three months of age. In order to do this, the heritability of 3-month weight will be established first. Following this, two questions will be addressed:

1. To what extent do the genes (or environmental factors) that influence appetitive characteristics during the first 3 months also influence weight at 3 months?
2. To what extent do shared genes (or shared environmental factors) account for the phenotypic associations between the appetitive traits and weight?

10.3. Methods

10.3.1. Establishing the heritability of 3-month weight SDS

Heritability analyses were conducted on 3-month weight SD scores that had been residualised for sample sex-effects and exact age in months (and days) when the weight measure was taken. The same sensitivity analyses that were conducted for the appetitive traits (described in Chapter 8, section 8.3.1.) were conducted for 3-month weight SD scores[82]. The same results that were obtained for the BEBQ scales were observed for 3-month weight SDS: estimates were unaltered by additional adjustment for gestational age, or removal of infants born before 34 weeks gestation, indicating that gestational age did not influence heritability and no adjustments relating to this factor were needed; removal of infants with reported feeding problems led to a slightly lower unique environment estimate that was outside the 95% confidence interval of the full sample with the standard adjustments but the difference was small (25% versus 33%), and removal of all infants with any reported feeding problem would have led to a sample that was almost half the

[82] Sensitivity analyses were conducted to ascertain whether heritability differed with and without infants with reported feeding problems, or with and without infants born before 34 weeks of age, or following additional adjustment for gestational age as a continuous measure (as well as age in days when the weight measure was taken and sex). If sample exclusions or additional statistical adjustment led to increases or decreases in the genetic or environmental estimates that were outside the 95% confidence interval of the whole sample using standard adjustment only, the additional adjustment or exclusion was deemed significant.

original size (*n*=2561 from *n*=4214). For these reasons problem-feeders were included and only the standard adjustments for age and sex were used[83].

The univariate heritability of weight was estimated using within-pair within-trait intraclass correlations, and standard ACE covariance modelling (described in Chapter 4, sections 4.5.1 and 4.5.2) as there was no evidence of genetic dominance from the intraclass correlations. As in Chapter 8, the fit of more parsimonious sub-models (AE, CE and E models) were tested using the LRT, AIC and BIC.

10.3.2. Identifying shared pathways underlying appetite and weight

All heritability analyses were conducted on BEBQ scale scores and 3-month weight SD scores that had been residualised for age[84] and sex effects, using the method described in Chapter 8. In keeping with Chapters 7 and 9, scores for 'slowness in eating' and 'satiety responsiveness' were reversed so that higher positive correlations indicated that greater appetite avidity was associated with higher weight, to aid interpretation of the results.

10.3.2.1. Twin correlations

Cross-twin cross-trait intraclass correlations were calculated for 3-month weight SDS and every BEBQ scale (and 'appetite size' in order to obtain an overall picture of shared pathways underlying appetite and weight more generally) to explore the shared heritability of 3-month weight and each trait independently. For every combination of 3-month weight and appetitive trait there were two cross-twin, cross-trait correlations – 3-month weight in twin 1 correlated with appetitive trait in twin 2, and appetitive trait in twin 1 correlated with 3-month weight in twin 2. These were compared to the phenotypic correlations that were

[83] The twin correlations for the sensitivity analyses for 3-month weight SDS are shown in Appendix 7.1, and the results from the covariance model-fitting sensitivity analyses are shown in Appendix 7.2.
[84] For the BEBQ scores 'age' refers to the age of the infants when the parents completed the questionnaire, for 3-month weight SDS 'age' refers to the exact age of the infant on the day the weight measure was taken.

calculated using Pearson's Product Moment correlation coefficients[85], to ascertain if there were indications of genetic influences common to 3-month weight and each appetitive trait. The twin correlations were performed in SPSS version 15 for Windows.

10.3.2.2. Covariance model-fitting

A multivariate saturated model was run including 3-month weight SDS and the appetitive traits in order to provide a comparison for the multivariate genetic models. Three multivariate models were run including the appetitive traits and 3-month weight SDS to examine the shared genetic and environmental influences on the appetitive traits and 3-month weight SDS, including a Correlated Factors Model, an Independent Pathway Model and a Common Pathway Model. Standard ACE models were run because there was no evidence of genetic dominance in the univariate analyses on the appetitive traits (presented in Chapter 8) or 3-month weight SDS (presented below in section 10.4). Each full model was compared to the saturated model and to each less constrained model within which it was nested (i.e. the Independent Pathway Model and the Common Pathway Model were compared to the Correlated Factors Model, and the Common Pathway Model was compared to the Independent Pathway Model). More parsimonious sub-models of the best-fitting Correlated Factors Model were tested against the full ACE model by systematically dropping combinations of parameters[86] (e.g. all of the genetic correlations were dropped collectively), and the goodness-of-fit of competing sub-models assessed.

Selection of the best-fitting model was based upon BIC changes from fuller models. As discussed in Chapter 9, BIC may be more informative when identifying the best-fitting and most parsimonious model from a number of competing models with large numbers of parameters and a large sample size. The covariance modelling was conducted using Mx

[85] Spearman's rho was also used to check the correlations between 'enjoyment of food' and 3-month weight SD scores because it was not normally distributed but the results were the same so only Pearson's Product Moment correlation coefficients are reported. The correlation coefficients reported here include all of the twins, and were performed on scores residualised for age and sex effects, therefore they differ slightly to the correlation coefficients reported in Chapter Seven which included non-residualised scores.

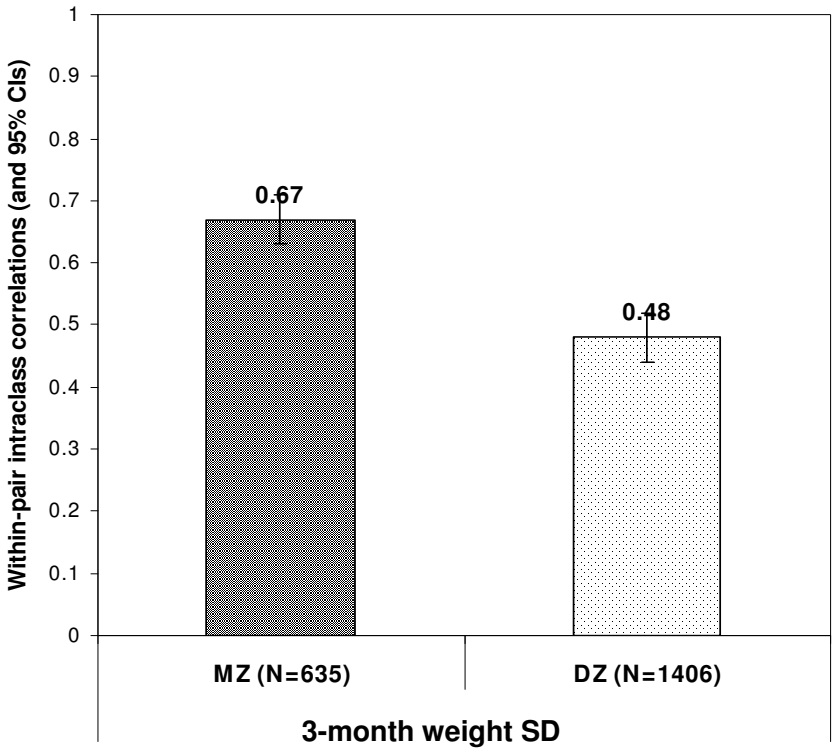
[86] E.g. all of the shared environment parameters were dropped, or all of the additive genetic parameters were dropped, or both sets were dropped, or all of the shared environment correlations were dropped, or all of the additive genetic correlations were dropped, or all of the unique environment correlations were dropped, or combinations of more than one set of correlations were dropped at the same time.

Maximum-Likelihood Structural Equation Modelling Software (version 32; Virginia Commonwealth University, Richmond, VA).

10.4. Results – establishing the heritability of 3-month weight SDS

A large number of infants (*n*=4214) had known zygosity and data for 3-month weight SDS. The twin pair intraclass correlations for 3-month weight SDS are presented graphically in Figure 10.1 for MZs and DZs. MZ correlations were higher than DZ correlations indicating a genetic contribution to weight variation, although the difference observed was somewhat smaller than the differences shown for the appetitive traits, suggesting that heritability is lower for weight.

Figure 10.1. Within-pair intraclass correlations (and 95% confidence intervals) for 3-month weight SD scores by zygosity



Abbreviations: MZ, monozygotic; DZ, dizygotic.

The parameter estimates from the covariance model-fitting analyses are shown in Table 10.1. The goodness-of-fit statistics for the univariate covariance models for 3-month weight SDS are shown in the tables detailing the sensitivity analyses in Appendix 7.2[87]. All of the goodness-of-fit statistics were in agreement that the best-fitting model for 3-month weight SDS included all three components. This was not surprising given that each component of variance played a fairly equal role – heritability was moderate at 37%, and the shared environment and unique child environment had similar effects estimated at 30% and 33% respectively.

Table 10.1. Parameter estimates (95% confidence intervals) for 3-month weight SDS (*n*=4214)

Model	Additive Genetic Effect (a^2)	Shared Environment Effect (c^2)	Non-shared Environment Effect (e^2)
ACE	**0.37 (0.27-0.47)**	**0.30 (0.21-0.38)**	**0.33 (0.30-0.37)**
CE	-	0.54 (0.51-0.57)	0.46 (0.43-0.49)
AE	0.70 (0.66-0.73)	-	0.30 (0.28-0.34)
E	-	-	1.00 (1.00-1.00)

Model: CE, AE and E models are nested within the full ACE model. The ACE model dissects the phenotypic variance into a^2, c^2 and e^2; the CE model drops the a^2 parameter and assesses the variance explained by the c^2 and e^2 parameters only; the AE model drops the c^2 parameter and assesses the variance explained by the a^2 and e^2 parameters only; the E model drops both the a^2 and c^2 parameters and assesses the variance explained by e^2 only. The most parsimonious model is bolded.

[87] The models in the top row of the table show the goodness-of-fit statistics for the analyses presented here, which included the whole sample with standard adjustments only.

10.5. Results –shared pathways underlying appetite and weight

10.5.1 Twin correlations

The cross-twin cross-trait correlations between the appetitive traits of the BEBQ and 3-month weight SDS are shown in Table 10.2, with the phenotypic correlations alongside for comparison. As can be seen the phenotypic associations between 'enjoyment of food' and 3-month weight SDS, and between 'food responsiveness' and 3-month weight SDS were small (0.14 and 0.10 respectively). Moreover, the MZ and DZ cross-twin cross-trait correlations between these traits and 3-month weight were approximately zero (and not significant), indicating that there was not enough cross-covariation to model, perhaps as a result of the small phenotypic associations. The small phenotypic associations and the lack of cross-covariance indicated that these two traits should not be included in the multivariate covariance model. The finding in Chapter 7 that these two traits are not related to 3-month weight independently of 'slowness in eating', 'satiety responsiveness' and 'appetite size' further highlighted that it was unnecessary to include them in the model as well.

'Slowness in eating', 'satiety responsiveness' and 'appetite size' had slightly higher phenotypic associations with 3-month weight SDS (0.19 to 0.29). Furthermore, in each case the MZ cross-twin cross-trait correlations were small but significantly different from zero, and about half the size of the phenotypic correlations, while the DZ cross-twin cross-trait were not significantly different from zero. This pattern of results indicates that for these appetitive traits, common genes but not common shared environmental influences, are contributing to the observed phenotypic correlations with 3-month weight SDS, and they indicate that shared genes could be accounting for up to half of the observed phenotypic associations.

Table 10.2. Cross-twin cross-trait intraclass correlations and phenotypic correlations of appetitive traits & 3-month weight

BEBQ Scale[a] & 3-month weight SDS	Twin[b] and Scale[a]	ICC[c] (95% Confidence Interval)		Phenotypic correlations[e]
		MZ[d]	DZ[d]	
'enjoyment of food' & 3-month weight SDS	Twin 1 EF * Twin 2 weight	**0.03** (-0.05, 0.10)	**-0.05** (-0.10, 0.00)	**0.14**
	Twin 2 EF * Twin 1 weight	**-0.01** (-0.09, 0.08)	**-0.05** (-0.10, 0.00)	
'food responsiveness' & 3-month weight SDS	Twin 1 FR * Twin 2 weight	**-0.01**(-0.09, 0.07)	**-0.05** (-0.10, 0.00)	**0.10**
	Twin 2 FR * Twin 1 weight	**-0.02**(-0.10, 0.06)	**-0.05** (-0.10, 0.00)	
'slowness in eating' & 3-month weight SDS	Twin 1 SE * Twin 2 weight	**0.10** (0.02, 0.18)	**-0.01** (-0.06, 0.04)	**0.19**
	Twin 2 SE * Twin 1 weight	**0.08** (0.01, 0.16)	**0.00** (-0.05, 0.05)	
'satiety responsiveness' & 3-month weight SDS	Twin 1 SR * Twin 2 weight	**0.09** (0.01, 0.17)	**-0.05** (-0.10, 0.00)	**0.20**
	Twin 2 SR * Twin 1 weight	**0.09** (0.01, 0.16)	**-0.05** (-0.10, 0.00)	
'appetite size' & 3-month weight SDS	Twin 1 AS * Twin 2 weight	**0.15** (0.07,0.22)	**-0.01** (-0.06, 0.05)	**0.29**
	Twin 2 AS * Twin 1 weight	**0.08** (0.01,0.16)	**-0.01** (-0.07, 0.04)	

[a] BEBQ, Baby Eating Behaviour Questionnaire; EF, 'enjoyment of food'; FR, 'food responsiveness'; SE, 'slowness in eating'; SR, 'satiety responsiveness'; AS, 'appetite size'.
[b] Randomly allocated twin (1 or 2) and scale used in the cross-twin cross-trait correlation.
[c] ICC, intraclass correlation.
[d] MZs, *n*=624-631 pairs; DZs, *n*=1397-1407 pairs.
[e] Pearson's product-moment correlation coefficients.

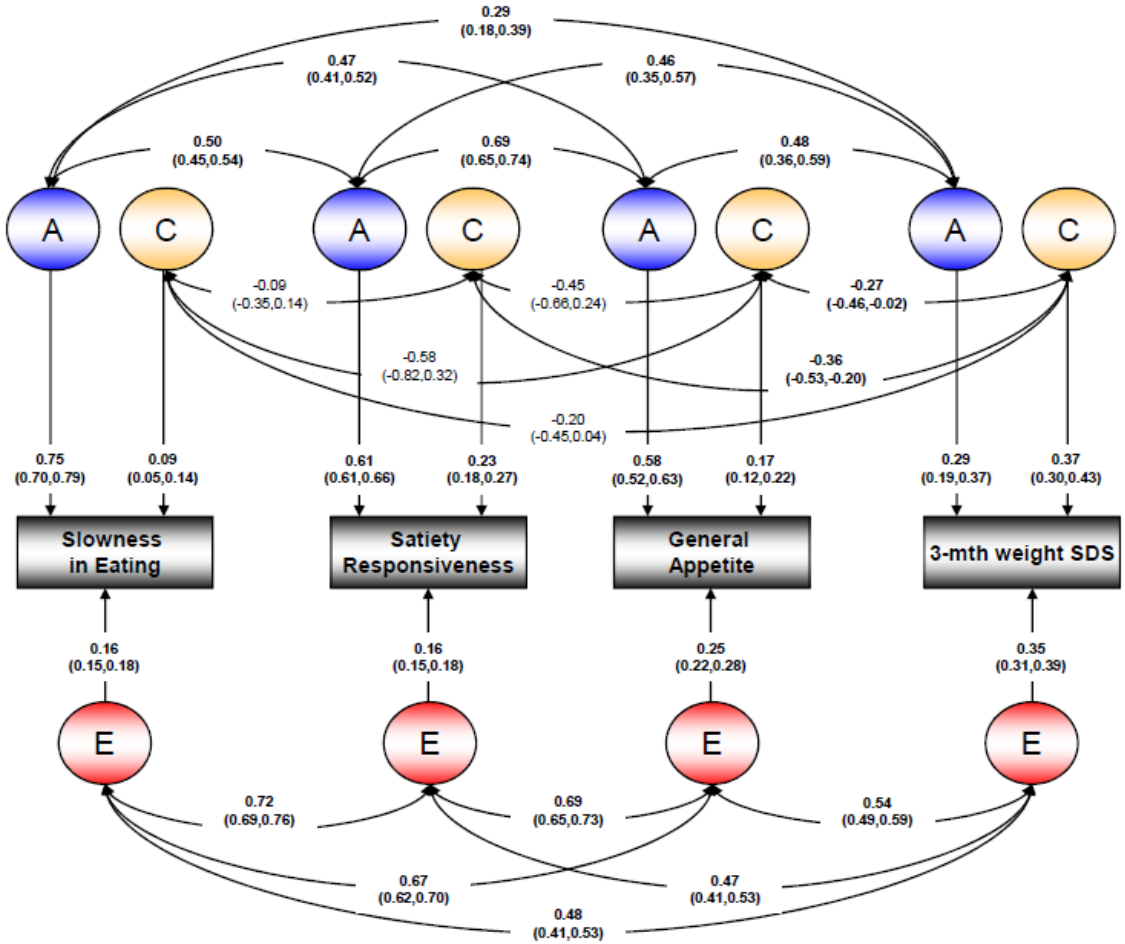
10.5.2 Covariance model-fitting

10.5.2.1. Selection of the best-fitting multivariate model

The multivariate models included ‘slowness in eating’, ‘satiety responsiveness’ and ‘appetite size’ along with 3-month weight SDS. The goodness-of-fit statistics for the different models tested are shown in Appendix 7.3. All of the full models provided an adequate fit to the data compared to the saturated model, according to BIC. The Correlated Factors Model had the lowest BIC value of the three models – the value was substantially lower than the value of the Common Pathway Model (-35.933) providing ‘very strong’ evidence for the less constrained model, and somewhat lower than the BIC value for the Independent Pathway Model (-5.246) providing ‘positive’ to ‘strong’ evidence that the Correlated Factors Model represents the data better than the Independent Pathway Model. The Correlated Factors Model was therefore preferred.

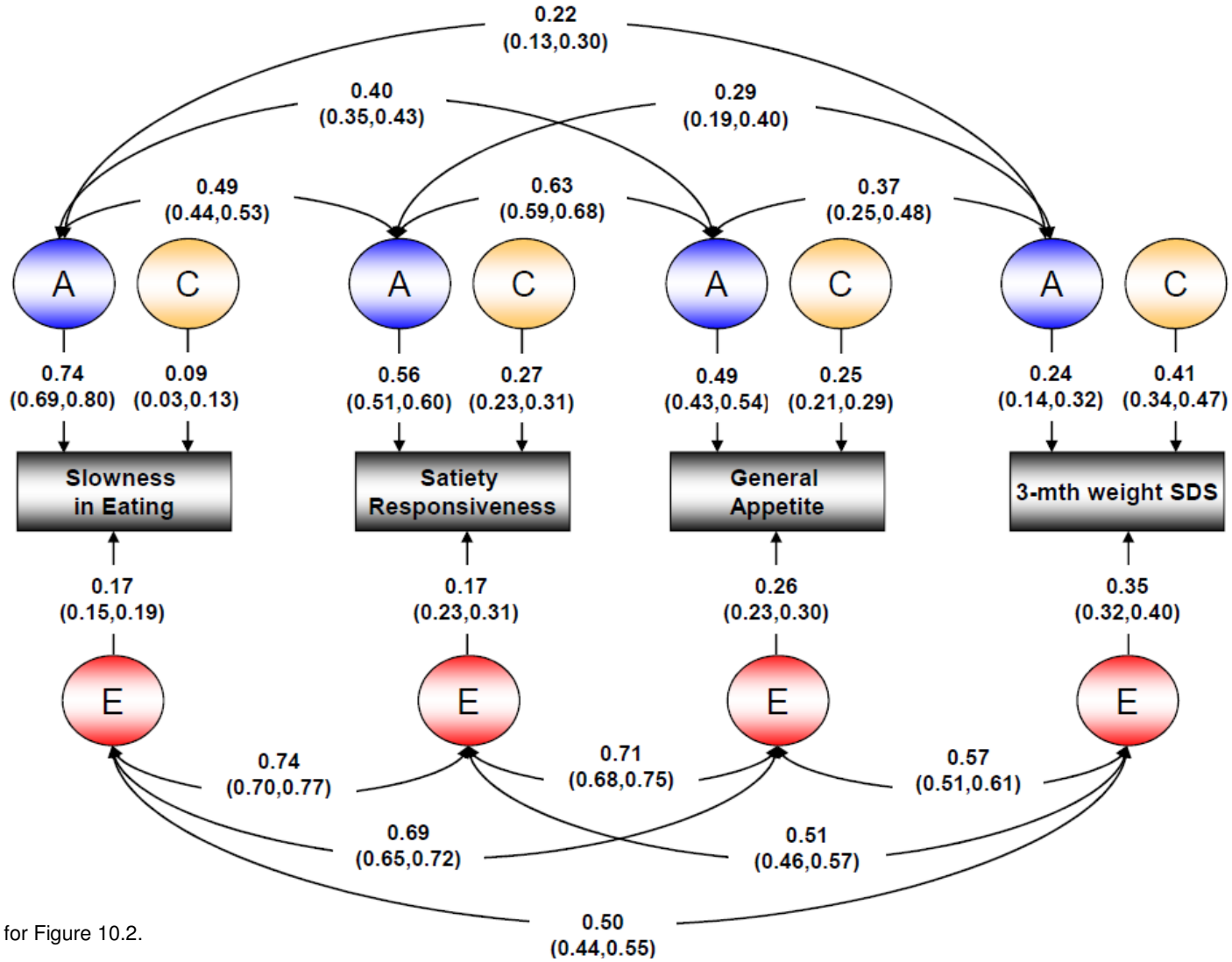
A more parsimonious sub-model was identified that dropped all shared environment covariances between the three appetitive traits and 3-month weight SDS. This was in keeping with the cross-twin cross-trait correlations that indicated no role for common shared environmental effects, and made empirical sense given the small amount of variance explained in the BEBQ scales from shared environmental factors in the univariate analyses reported in Chapter 8 (0%, 12% and 3% respectively). The parameter estimates from the full model are shown in Figure 10.2 and those from the more parsimonious sub-model are shown in Figure 10.3. Only the results from the more parsimonious sub-model are discussed in the following section.

Figure 10.2. Full ACE Correlated Factors Model showing the shared genetic and environmental influences on appetitive traits and 3-month weight SDS



Path diagram showing the genetic and environmental influences on appetitive traits and 3-month weight SDS for one child using a Correlated Factors Model. The rectangular boxes represent the measured phenotype (BEBQ scale or weight). The circles indicate latent influences on the measured phenotype which include additive genetic effects (A), shared environmental effects (C) and unique environmental effects and error of measurement (E). The straight single-headed arrows show the causal paths, and the squared path coefficients on each causal path indicate the total variance explained in each eating behaviour and weight by A, C and E. The curved double-headed arrows show the genetic, shared environment and unique environment correlations between the traits which can range between 0 and 1. Significant parameter estimates for which the 95% confidence interval did not include zero are bolded.

Figure 10.3. Preferred Correlated Factors sub-model showing the shared genetic and environmental influences on appetitive traits and 3-month weight SDS



See footnote for Figure 10.2.

10.5.2.2. Multivariate findings from the preferred sub-model

A Correlated Factors Model was preferred that included genetic covariance and unique environmental covariance, but no shared environmental covariance between 3-month weight SDS and the three appetitive traits ('slowness in eating', 'satiety responsiveness' and 'appetite size'), in keeping with the indications from the twin correlations. This suggests that during infancy there are common genetic influences and common unique environmental influences that all contribute significantly to the covariation between the three appetitive traits and 3-month weight SDS, while common shared environmental factors do not play a role (or the size of the influence is too small to be detected with this sample size).

Some observations are noteworthy (Table 10.3). Firstly, common genetic influences and common influences of the unique child environment appeared to play a fairly equal role in driving the observed phenotypic associations between the appetitive traits and weight, as indicated by the bivariate heritability estimates between 3-month weight SDS and the appetitive characteristics (41% to 45%), and the corresponding bivariate unique environment estimates (50% to 57%). Common genes did not appear to play a more important role for one pairwise correlation over another.

Secondly, while common genetic influences and common unique environmental influences appeared to play an equal role in explaining the phenotypic associations observed, the genetic correlations (r_A) were fairly modest (0.22 to 0.37), and were significantly smaller (as shown by the 95% confidence intervals that did not overlap) than the unique environment correlations (r_E) in each case (0.50 to 0.57) (see Table 10.3 and Figure 10.3). This suggests that most of the genes that influence 3-month weight are independent of the genes that influence the appetitive traits, but that the small number of shared genes account for about half of the observed associations. On the other hand, about half of the unique environmental influences that influence appetite, also influence 3-month weight SDS, as indicated by the magnitudes of the unique child environment correlations.

Lastly, once covariation between 3-month weight SDS and the three traits had been taken into account in the multivariate model, the heritability estimates obtained were somewhat lower for all of the phenotypes than those observed in the univariate analyses on appetite (Chapter 8) and 3-month weight. On the other hand, the effect of the shared environment increased fairly sizeably, probably as a result of increased power to detect smaller effects, indicating the benefit of taking a multivariate approach.

Table 10.3. Parameter estimates (95% confidence intervals) for appetitive traits & 3-month weight SDS from ACE multivariate Correlated Factors Model constraining shared environment covariances to be zero

Variables	*r*	Bivariate A	Bivariate E	r_A	r_E
'slowness in eating' & 3-mth weight SDS	**0.22** (0.18,0.25)	**0.42** (0.26,0.54)	**0.58** (0.46,0.74)	**0.22** (0.13,0.30)	**0.50** (0.44,0.55)
'satiety responsiveness' & 3-mth Weight SDS	**0.23** (0.20,0.27)	**0.45** (0.32,0.56)	**0.55** (0.44,0.70)	**0.29** (0.19,0.40)	**0.51** (0.46,0.57)
'appetite size' & 3-mth Weight SDS	**0.30** (0.27,0.34)	**0.41** (0.28,0.52)	**0.59** (0.48,0.71)	**0.37** (0.25,0.48)	**0.57** (0.51,0.61)

Abbreviations: *r*, phenotypic correlation derived from covariances; Bivariate A, bivariate heritability (contribution of common additive genetic effects to the phenotypic correlation); Bivariate E, bivariate unique environmental effects (contribution of common unique environmental effects to the phenotypic correlation); r_A genetic correlation; r_E, non-shared environmental correlation.

10.6. Discussion

10.6.1. Summary of findings

There has been a growing interest in the idea that genes are influencing weight (and growth rate in early life) through behavioural mechanisms that interact with environmental exposures in the determination of weight. This study sought to test this hypothesis more directly by exploring if there are shared genetic pathways underlying appetitive characteristics measured in the BEBQ during the first three months of life, and weight at three months. Two questions were addressed by this study and the results are discussed within the context of each, below.

10.6.1.1. To what extent do the genes (or environmental factors) that influence appetite at 3 months also influence weight at 3 months?

The results of this study provide support for the idea that there are common genes underlying appetitive characteristics and weight at the beginning of life. The genetic correlations were modest for all of the appetitive traits included in the model suggesting that about a quarter to a third of the genes that influence 'slowness in eating', 'satiety responsiveness' and 'appetite size' also influence weight (r_A = 0.22, 0.29 and 0.37 respectively). This makes good empirical sense given the modest magnitude of the phenotypic associations, and the fact that there are many unrelated processes that are involved in appetite regulation and weight that are likely to arise from highly specific genetic variants. Nevertheless there also appear to be a substantial number of genetic influences that are common to both. These findings accord well with the only study to have explored shared pathways between appetitive traits measured using the TFEQ and weight in adults. Keskitalo and colleagues (2008) found genetic correlations of 0.29 for 'uncontrolled eating' and BMI, and 0.51 for 'emotional eating' and BMI, suggesting that a third to half of the genes that influence appetitive traits also influence adiposity in adults.

These findings fit in well with our existing knowledge of molecular genetic variants that are known to influence weight in adults and children. There has been a recent surge in

genome-wide association studies (GWAS) identifying single nucleotide polymorphisms (SNPs) that relate to indices of adiposity, since the discovery of *FTO* in 2007. In response to the burgeoning research base, an international consortium (the Genomewide Investigation of ANTHropometric measures, GIANT) has been assembled to pool results from these studies so that SNPs that show robust associations with weight might be identified, and two meta-analyses have been published (Speliotes et al., 2010; Willer et al., 2009). The most recent update included explorations of ~2.8 million SNPs in >123,000 individuals, and concluded that 32 loci clearly relate to adiposity (Speliotes et al., 2010). About one third of the variants map near important hypothalamic regulators of energy balance (e.g. *TMEM18, KCTD15, SH2B1, GNPDA2, NEGR1, BDNF* and *POMC*, as well as *FTO* and *MC4R*) and one encodes a receptor of gastric inhibitory polypeptide (*GIPR*), suggesting that a number of the loci identified are likely to be operating through appetitive mechanisms centrally and peripherally. Collectively, the 32 variants explained a tiny amount of variance in BMI (1.45%, with 0.55% explained solely by *FTO*), but it is possible that some of the genes will have a larger effect size on appetite if they influence adiposity through this pathway, as appetitive characteristics may be endophenotypes that sit closer to the gene effects. The group estimated that it is likely that an additional 284 loci of similar effects might be identified with larger and more carefully designed studies, and it is reasonable to assume that a substantial proportion of these will exert their effects on weight through appetitive characteristics. As far as I am aware, other than *FTO* and *MC4R,* none of the 32 variants have yet been related to appetitive characteristics in any age group, and *MC4R* and *FTO* have not been explored in early life. We have collected DNA from the Gemini infants and are measuring a number of variants that may relate to both appetite and weight during this early period of life.

In contrast to the modest genetic correlations there were fairly high unique environmental correlations (0.50 to 0.57), suggesting that at least half of the environmental factors unique to each child that were shaping appetite were also influencing adiposity at this early age. Possible candidates this early on in the life cycle could include those suggested in Chapter 9 as potential shapers of appetite. An example would be intrauterine disparity in nutrient acquisition as a result of differential blood supply across the placenta(s) or cord(s) (affecting both MZs and DZs) (van Baal & Boomsma, 1998; Loos et al., 2005a), an extreme case of which is seen in twin-to-twin transfusion syndrome where twins are born hugely discordant for weight (Lopriore et al., 2003). As discussed, foetal under- or over-

nutrition is hypothesised to lead to 'programming' effects such as epigenetic alteration to the hypothalamic circuits controlling energy balance such that appetite is upregulated, which in turn influences weight gain postnatally. Another possibility is differences in feeding method – bottle-feeding or breast-feeding one twin more than another may influence appetite and weight, although this relationship is not straightforward in this particular sample of infants, as shown in Chapters 6 and 7. The preferred model did not include any shared environmental effects that were common to appetite and weight; this is not surprising given the size of the shared environmental effects on the appetitive traits observed in the univariate analyses which ultimately limits the amount of available shared environment variance to model with weight.

The heritability of weight at 3 months was only modest (37%), at the lower end of estimates from other studies (Demerath et al., 2007; Levine et al., 1987). Twin studies tend to report lower estimates for weight heritability than family studies during the first few months as twins are still 'catching up' following growth restriction in utero (Bouchard, 2009).

10.6.1.2. To what extent do shared genes (or shared environmental factors) account for the phenotypic associations between appetite and weight at 3 months?

A key finding from this study was that in spite of the fairly modest genetic correlations, the genes that are common to both appetite and weight play a very important role in driving the phenotypic associations between them, with common genes explaining about half of the observed covariation. A consideration here is that the low heritability of weight at this age may have limited the amount of genetic covariance shared with appetite (Plomin et al., 2008). It is also possible that the importance of the common genetic influences driving both appetite and weight become more potent as the infants get older and become more independent with regard to their eating behaviour, being able to act in accord with their own appetitive desires and so allow the genes that influence their appetite to influence their weight to a greater extent. The only other study to explore shared genetic influences between appetite (measured using the TFEQ) and weight used adults (Keskitalo et al., 2008); it is noteworthy that in this older sample shared genes accounted for 81% to 100% of the phenotypic associations between BMI and appetite (despite modest genetic

correlations), showing that later in life common genes may entirely drive the phenotypic associations between appetite and adiposity.

In this study, effects of the unique environment that are common to both appetite and weight played an equal role to genetic factors in influencing the phenotypic associations. This seems surprising given the suggestion that more unique environmental factors than genetic factors are shared by appetite and weight (indicated by slightly higher environmental correlations than genetic correlations). It could be the case that although most of the unique child environmental factors that influence appetite also influence weight, the sum of these many environmental effects are no larger than the sum of shared genes in influencing covariation.

10.6.2. Implications for theory, practice and future research

There are some obvious implications for these findings. Of the 32 genetic variants found to relate to weight in the recent GIANT meta-analysis (Speliotes et al., 2010), those that appear to be involved in central or peripheral appetite regulation are good candidates to explore in relation to 'slowness in eating', 'satiety responsiveness' and 'appetite size' during infancy. If variants are found to relate to these appetitive traits, there are good grounds for targeting these characteristics in behavioural interventions that are designed to ameliorate weight gain or facilitate weight loss, early on in life. Understanding the function of these genes will also allow for the development of pharmacological treatments that may act at key regions of gene expression to help down-regulate appetite, and in turn reduce weight, although this may not be appropriate so early on in life. One of the limitations of the genome-wide association study method is that it is not hypothesis-driven, but hypothesis-generating – variants identified must be investigated further to elucidate their functional roles, and the BEBQ make help to clarify this from the beginning of life.

These findings also suggest that it would be useful to search for environmental factors that are unique to each child that influence both appetite and weight. If we can identify specific aspects of the unique child environment that are important shapers of appetite, then there is a reasonable probability that they will also relate to weight (a 50% to 57% chance, in line

with the unique environmental correlations). Differential programming effects in utero are likely, but these are very difficult to measure, especially for each twin separately. Factors in early post-natal life are easier targets, such as feeding method or illness. In Gemini we have collected measures for each child for a number of early life experiences and we will be able to explore how these contribute to appetite and weight covariation. Making use of MZs who are discordant for both appetite and weight will provide a powerful method of identifying unique environmental factors.

10.6.3. Strengths and weaknesses

As far as I am aware this is the first study to explore shared pathways underlying appetite and weight in infancy, and this is virtually uncharted in adults. These findings suggest that we should expect about a third of the molecular genetic variants identified that relate to weight to also relate to appetite, and vice versa. However, some limitations must be acknowledged. Firstly, this is a cross-sectional analysis and it could be the case that weight and appetite show a common genetic pathway because genes influence weight, and weight influences appetite. But the evidence from molecular genetic studies does not support this directional pathway. Rather the recent findings are suggestive of a behavioural susceptibility model of weight. Demonstrating genetic correlations between appetite and weight gain prospectively would strengthen the evidence.

Secondly, the phenotypic correlations were small which compromises the reliability of the genetic and environmental correlations. The sampling errors for estimates of genetic and environmental correlations are relatively large when phenotypic correlations are low (Falconer & MacKay, 1996), although this is less of an issue with large datasets such as the one used here (Falconer & MacKay, 1996). Lastly, twins are born smaller and so tend to experience ‘catch up’ growth more frequently than singletons; due to the different growth patterns of twins and singletons early on in infancy the architecture of shared genetic and environmental pathways with appetite may be slightly different to that of singletons. It would be useful to include siblings in the model as well, in order to show that the results remain the same, or to adjust for the differences. This will be possible in Gemini

as we are currently collecting BEBQ data on younger siblings. In addition, replicating these findings in the Gemini twins when they are in early or middle childhood (by which time the effects of being born smaller have ‘worn off’) would strengthen their credibility.

CHAPTER 11. STUDY 6: CASE STUDY OF S – AN IN-DEPTH EXPLORATION OF EXTREME EATING BEHAVIOUR

11.1. Background

An advantage of the quantitative methods used in Studies 1 to 5 is that they permit rigorous testing of hypotheses. A disadvantage is that much of the information is lost. Qualitative approaches may serve to enrich quantitative data by providing more in-depth understanding of the questions of interest, and more detailed data (Miles & Huberman, 1984; Patton, 1980). In particular, in-depth exploration and characterisation of extreme eating behaviour may help to build on existing theory or generate hypotheses, and can increase understanding of these phenomena by providing 'real life' contextualised information about the manifestation of these traits and the consequences for the individual child and other members of the family (Eisenhardt, 1989; Hamel, 1993; Stake, 1995; Yin, 1984). I was presented with the opportunity to study in detail an infant demonstrating a highly upregulated appetite, to compliment the findings in this thesis with richer, contextual information.

11.2. Study aim

The aim of the present case study is to provide an in-depth exploration of an infant who is displaying eating behaviours indicative of an extremely avid appetite.

11.3. Methods

Following the broadcasting of a Horizon documentary that featured research from our group on the relationship between child eating behaviour and weight, a mother was struck by how the description of the 'obesogenic' eating styles characterised her own infant who

was extremely food responsive, demonstrated exceptionally high levels of reward from eating, ate with a very rapid pace, and appeared to have poor satiety sensitivity. The mother contacted our research department describing her child as displaying an 'aggressive' and 'ferocious' appetite, and she was invited to come for an interview so that we might discuss her daughter's eating behaviour in more detail and plan a home visit to study the child's eating behaviour in a naturalistic environment.

11.3.1. Interview with the mother and initial assessment of S's eating behaviour and weight

In May 2009 the mother (KB) came into our unit without her daughter (S) to be interviewed at length during which she was asked to describe S's eating behaviour in detail, discuss other similar instances in the family, talk about the early presentations of these traits, and discuss how she currently manages it. Before coming in to see us, we sent her a height chart with detailed instructions so that S's height could be measured the day before the interview, as well as her weight. Her current weight and height were used to determine her BMI and BMI centile for her age and sex based on British 1990 reference data (Freeman et al., 1995), using the LMSgrowth macro for Microsoft Excel (Cole, 2009). Her mother also provided anthropometric information on her daughter from her red book (health record) so that we could look at growth patterns from birth. In addition, the mother had completed the CEBQ (Wardle et al., 2001b) and a 3-day food diary prior to the interview so that we might compare her eating behaviours and food intake to her estimated energy requirements, and to current recommendations. The dietary data was coded by a dietitian at the Health Behaviour Research Centre, using the Nutrient Analysis programme in Microdiet (Downlee Systems Limited, 2000).

11.3.2. Home visit

Following the interview I visited S and her mother (KB) at home in June 2009 to observe her eating behaviour in a real life setting. An ad libitum meal was laid out that consisted of a variety of foods (ham and cream cheese sandwiches, egg sandwiches, cheddar cheese sandwiches, ham, sausages, eggs, cheese sticks, potatoes, savoury biscuits, sweet

biscuits, orange, apple and banana), presented as a 'teddy bear's tea party'. Before the home visit I sought advice from a dietitian about the type and amount of food to ensure that a variety of food groups were on offer and that more than an entire day's energy requirement was available. Following consultation with the dietitian, I telephoned S's mother and asked her to provide the list of foods that we had agreed. Upon arrival at the home I made up the sandwiches and placed similar food groups together on separate plates. The plates were weighed before and after the meal so that the calories and weight of food consumed could be approximated later. The food provided contained over 2000 kilocalories (kcal). A video camera was set up in the corner of the room so that S's eating styles might be studied in detail following the visit. A dietitian at the Health Behaviour Research Centre calculated the approximate number of calories consumed from the changes in weight to the food plates.

11.3.3. Follow-up

After the home visit I wrote an email to S's mother summarising my observations during the meal. I have remained in regular contact with her mother to monitor S's progress and offer advice when sought.

11.4. Results

11.4.1. Measures taken before the interview – eating behaviour, food intake and weight

S's weight was 13.11 kg and she was 81 cm tall, giving her a BMI of 19.97, placing her on the 98th centile for her age (exactly 18 months) and sex, and putting her in the obese category. Her mother, however, described her as "not overweight yet". During the three day period over which her diet was recorded in the food diary she consumed an average of 1080 calories per day, estimated as 87% of her daily energy requirements calculated to be 1245 calories / day based on 95 kcal per kg of body weight (Department of Health, 1991). There were, however, some differences in intake over the three days – on day 1 she consumed the least taking in only 697 kcal, on day 2 she consumed 1087 kcal and on

day 3 she ate double the amount on day 1 at 1456 kcal. Protein is the only macronutrient for which there are daily recommendations at her age (1 to 3 years), suggested as 14.5 grams (Department of Health, 1991); for this nutrient she consumed 434% of her recommended daily allowance at 63 grams. Other than this, no aspects of the food intake in the diet diary concerned the dietitian or stood out as unusual, although she commented that her diet overall was healthier than most diet diaries of toddlers that she had reviewed, with a large proportion of her intake being taken up with fruits and vegetables, lean meats and whole grains.

On the other hand, her appetite, measured using the CEBQ, stood out as extreme. She had the maximum score for 'enjoyment of food' (5) and a very high score for 'food responsiveness' (4.8) highlighting that, in the mother's opinion, S always demonstrated the behaviours indicative of these traits. She obtained the minimum score (1) for both 'satiety responsiveness' and 'slowness in eating' showing that she always consumed her food at an extremely fast pace and never showed any behavioural signs of fullness as far as her mother could observe. She also scored as low as possible on 'fussiness' (1), being happy to eat any kind of food. Her mother did not complete the two scales relating to emotional eating because she felt S was too young for the answers to be meaningful. These scores indicated that S was extremely food responsive, enjoyed eating to a very large extent, was happy to eat any type of food on offer, ate rapidly and appeared to show no satiety sensitivity.

11.4.2. Interview

The interview lasted for just over one hour during which KB discussed her daughter's early appetite for milk and later for food, her concerns about her growth, the strategies that are currently in place to manage her eating behaviour, and her concerns about management issues in the future. She also discussed other members of the family, and S's general development in other areas. Her main points are summarised under the separate headings below. KB and her husband (S's natural father) both work full time in professional jobs – she is a management consultant in the city of London, and he is a political advisor to the Labour Party. They live in an apartment in West London. They are both in their mid 30s.

11.4.2.1. Appetite and growth during the milk-feeding phase

S was breast-fed exclusively for the first six months of life, in line with recommendations at the time. Her mother felt that during this phase her appetite appeared normal, although she conceded that it is difficult to know how much a breast-fed infant is consuming. Nevertheless, she pointed out on S's growth chart that she had started to cross percentiles even during the early breast-fed period – she was born on the 50th percentile and had reached the 98th by 7 months, and has remained there, suggesting that overfeeding had begun soon after birth.

It was not until she started bottle-feeding S with formula milk at around six months (the same time that she was weaned) that KB started to become concerned about her weight gain. It had crossed her mind that she was giving her too much formula milk, but she always followed the instructions provided by the product regarding the ratio of powder to water, and the frequency with which she should be feeding S.

11.4.2.2. S's response to solid food

S responded very well to being weaned insofar as she liked all foods offered to her (including fruits and vegetables), never turned any food away, and was not difficult to feed. However, by 9 months of age it had become apparent that S's appetite and behaviour around food was abnormal. Her mother noticed that she would want food as soon as she saw it, if refused it would become angry and aggressive (there could be no food on show in the house), and she would "gobble" food once it was given to her. This style of eating has continued and she showed an increasingly ferocious appetite as she grew older. When food is available to her she eats so quickly and puts so much of it into her mouth in one go that she sometimes retches at the start of meals. This behaviour is exacerbated by the presence of other children or adults who are also eating, and KB senses that this is due to S wanting to make sure that she can "get more" in the face of competition from others, and take "ownership" of the food that is available.

Other people have also noticed S's behaviour and have broached the issue with her mother. She was cared for by a child-minder from 9 months of age and joined a nursery when she was 16 months old. The first child-minder became increasingly concerned about her appetite and continually brought it up with KB, describing her as aggressive and difficult to deal with around food. More recently, the nursery that she attends wrote a letter to KB voicing their concerns, which she gave to me to read. They described her as eating a large portion of food at mealtimes in comparison to other children under five, and they believe she would continue to eat more were she given the chance; she also has a tendency to put all the food into her mouth at once until she retches and chokes. S is described in the letter as being "obsessed with food" and the nursery staff feel that it is a problem that needs addressing while she is young, before it gets out of control. They listed five instances of her behaviour around food that have given them cause for concern at the nursery:

1. "S will cry when she sees food indoors and out, wanting it. This even happens if she has just eaten a two course meal."
2. "After eating her food she will then cry for the other children's food, and if in her reach will try and take it."
3. "S associates the kitchen and fridge with food and if you go into either and don't give her food she will cry."
4. "If S sees food on the floor while outdoors she will try and eat it, and when told not to will cry."
5. "If not encouraged to slow down when eating S would finish a meal within five minutes".

Just prior to the interview, there had been an upsetting incident at the nursery. The staff allowed S to eat as much food as she wanted without limiting her intake. She continued to eat large quantities of food until she started to vomit, and quickly became very distressed.

11.4.2.3. Current management and future concerns

KB is desperate to find strategies that will attenuate S's appetite so that excessive weight gain might be avoided, but this is starting to prove difficult already. At the present time, because of her young age, it is possible to control her food intake to some extent, but her

mother worries about the future when S is older and can make independent decisions around food. Currently, all of S's meals are home-cooked and she is given a balanced and healthy diet, and this happens both at home and at nursery. KB makes a concerted effort to ensure that S eats very well, giving her plenty of fresh fruits and vegetables, and wholegrain foods, and she has given the nursery staff strict instructions about the types of foods to give her. S eats porridge for breakfast at home everyday with her parents – she eats it very quickly and then wants that of KB's and her fathers, so KB gives her a whole apple to eat after her porridge which takes her up to an hour because it requires a lot of biting and chewing, and after she has finished it she tends not to ask for any more food.

During the week she eats lunch at her nursery with other children. The staff have been teaching S to eat more slowly, and in particular, to take in smaller pieces of food and chew it properly. They have commented that she is making progress in this area and although she will still put all of the food in to her mouth, she is beginning to chew it more efficiently. Other strategies that the staff have used are to involve her in preparing meals, and in clearing up afterwards to distract her from wanting more food following the meal, and to help her to understand that food is not simply about immediate consumption. Dinner is eaten with her parents at home – they must eat at the same time as S (6pm) because she becomes angry and aggressive if she is aware that they are eating afterwards and she is not allowed anymore food. The same problems occur with dinner as with breakfast in that she eats her meal very quickly and then wants her parents' food. KB finds evening meals very stressful; they tend to end with S having a tantrum because she has been denied more food or KB gives in to her and gives her some of her own meal. She described this time of day as "very hard". KB gives her lots of water to drink during the meal to slow her eating down and to help with her swallowing, and finishes by giving her a large carrot or an apple to eat that she knows will take her a long time.

KB emphasised how hard she and S's other caregivers work to control her eating behaviour. At the same time, she is worried that too much control is not good either, because when S gets older and can eat independently she may overcompensate when parental restriction is not possible, such as at school. In order to regulate S's eating behaviour at the moment without enforcing too much control, KB and S's father do not eat in front of her unless it is a meal in which she is partaking, and no food is ever on show in the home. In the future, KB plans to focus on S's eating speed rather than the quantity of

food that she wants to consume, because she believes that if she slowed down she would eat less overall. A strategy that she plans to focus on is to encourage her to put her fork and spoon down between bites. She will continue to give her foods that require chewing and effort to eat but are low in energy such as vegetables, and some fruits.

11.4.2.4. Family history

S's father and many members of his family were described as "struggling" with their weight. He himself is 1.85 metres tall (6 feet 1 inch) and weighs about 114 kgs (18 stones) giving him a BMI of 33.2, and placing him in the obese category. KB described him as "solid but not really big". In particular, she mentioned that his mother and sister are very overweight, commenting that his sister is "really big" in comparison to her brother. KB described him as having unhealthy eating patterns characterised by a healthy and normal-sized portion of porridge in the morning, but nothing throughout the course of the day until he returns home in the evening when he eats dinner followed by "lots more"; he likes to eat "rubbish" (e.g. crisps and other junk food) and is a "comfort eater", who has a tendency to binge on certain types of foods. She described her husband as very sensitive about his eating behaviour, and conscious of his weight, and he exercises regularly in order to try and counter the weight gained from his eating. He has wondered if his avid appetite is being caused by an undiagnosed medical problem. In contrast, KB is slim (although we did not ask for her height and weight to calculate her BMI), does not overeat and does not have a taste for the junk food that her husband craves. Her weight has been stable for a number of years so she no longer feels the need to weigh herself.

11.4.2.5. S's general development

All other aspects of S's development have been normal and she has no diagnosed medical disorder known to cause hyperphagia. She was standing from 9 months of age, walking from 14 months and is very physically active. She is a good sleeper, going to bed at 7pm and waking no earlier than 6 or 7am. She appears to be bright and sociable, her language is developing normally and she is interested in books.

11.4.3. Home visit

I arrived at the family home at about 11:30am on a Saturday about one month after the interview, in time to prepare the ad libitum meal to coincide with S's lunchtime. During the preparation of the meal S was very agitated and became somewhat aggressive in her attempts to obtain the food that was being put on to plates. She began to whimper and cry, and repeatedly jumped up at the kitchen surface to snatch the food. As she realised that she was not going to be allowed to get hold of the food she appeared angry and started to scream and throw a tantrum. Her mother became increasingly anxious by S's behaviour, and had to physically restrain her to keep her out of the kitchen. I found the preparation of the meal very difficult.

The meal was laid out on a large coffee table in the living room with a little chair for S and a number of her toys sitting in chairs around the table to participate in the 'teddy bears' tea party'. The food plates covered most of the table. It was possible for S to select any of the foods that she wanted, to eat as much as she desired, and to get up and move around the table if she wished to. Her mother and I stayed with her and pretended to pour cups of tea for the toys. As soon as the food was available to S she stopped crying.

S's eating behaviour during the meal was quite extreme. She began by putting an entire handful of ham into her mouth in one go, followed by a whole hard-boiled egg; she quickly became overwhelmed and began to gag and choke, and her mother gave her some water to drink and reminded her to chew the food before she tried to swallow it. Her eating continued in this vein, forcing as many handfuls of food into her mouth as she could fit, and filling both hands with food ready to eat once she had swallowed whatever was in her mouth. She seemed to chew very little before swallowing. Over the course of the meal she consumed 1107 kcal (517.31 grams), which was approximately all of her energy requirement for one day, and she ate for a total of 47 minutes, although she appeared to slow her eating in the second half of the meal. She did not consume all of the food provided. The kcals consumed from the various foods are shown in Table 11.1. S did not particularly favour one type of food over another, and was as interested in fruit as she was

in the sweet biscuits. She did, however, consume all of the protein foods but none of the savoury starchy foods.

Two behaviours stood out during the meal. Firstly, the speed with which she ate the food was extremely rapid, putting handfuls of food into her mouth in quick succession, and swallowing as quickly as possible. Secondly, she appeared to be possessive of the food – at some points during the meal when asked by KB if 'teddy' could have a biscuit she said 'no'. Figure 11.1 shows four still photographs of S's enthusiastic eating behaviour demonstrated during the ad libitum meal. Following the meal her stomach was distended, although she appeared happy and content.

Table 11.1. Amount of food (kcal and grams) consumed by S during the ad libitum meal

Foods consumed	Grams	Kcal
Fruit (banana, apple, orange)	190.75	103
Sweet biscuits (Foxes Butter Crinkles)	56.74	267
Mixed sandwiches (ham and cream-cheese, cheddar cheese, egg)	141.86	391
Protein foods (ham, sausages, cheddar cheese, egg)	127.96	346
Starchy foods (potatoes, bread, savoury biscuits)	0	0
Total	**517.31**	**1107**

Figure 11.1. Four stills of S eating during the ad libitum meal, taken from the videotape (minutes: seconds into the meal)

Still 1 (3:24): KB intervening to remind S to chew the food before swallowing after S became overwhelmed by putting an entire egg and a handful of ham in to her mouth and began to retch and choke.

Still 2 (4:50): S reaching for some fruit with a sandwich already in her mouth and a biscuit in one hand.

Still 3 (10:27): S putting a handful of ham into her mouth with a sandwich in her other hand.

Still 4 (17:10): S reaching for more biscuits with food in her mouth and an arm full of biscuits.

11.4.4. Follow-up

Immediately following the meal, KB was somewhat shocked and upset by the amount of food that her daughter had consumed, and the rate at which she had eaten it, although S wasn't sick after the meal. She asked for my advice as to what she could do to get S the help that she needed to address her avid appetite. After consultation with Professor Jane Wardle, I advised her to take S to her GP and explain her concerns, and to ask the GP to refer her to an endocrinologist in secondary care who specialises in childhood obesity to investigate if there is an underlying disorder that might be causing her hyperphagia. When I returned to work on Monday, the dietitian calculated the amount of food consumed by S, and I watched the videotape to remind myself of her eating style, and wrote an email to KB to summarise my observations so that she might take it with her to the GP.

S was seen by her GP the following week and referred to a group at Great Ormond Street Hospital. Initially the secondary care team were reluctant to organise an appointment for S because she was "too young" and in their opinion her obesity was not yet severe enough to warrant medical intervention. However, KB persisted and eventually she was seen. The group ran several tests but were unable to identify any endocrine abnormalities that might explain her hyperphagia; she was referred to a research group in Cambridge for genetic testing for major mutations known to cause early onset obesity and hyperphagia but is still awaiting the results. KB felt disappointed by the lack of a diagnosis and S was discharged from secondary care. It remains KB's responsibility to manage her daughter's eating behaviour. The family recently moved to a different part of the country and her new GP emailed me to seek advice about managing her appetite; we responded by providing him with details of the few successful interventions that have been published in the area, and our group will continue to support S and her mother in any way that we can.

11.5. Discussion

This case study illustrates how an extremely upregulated appetite in early childhood manifests itself in the distinctive eating behaviours described in Chapter 1, including very high food responsiveness, heightened subjective reward experienced from eating food, a

very rapid pace of eating, poor satiety sensitivity, and a willingness to eat anything. The child's eating behaviour was captured very well by her CEBQ scores for the corresponding scales. This case describes the challenge that these behavioural traits present for the parents of such children, and the weight gain that has already occurred so early on in the life cycle – despite huge efforts by those caring for her to manage her food demands, S is already obese at 18 months.

It is noteworthy that S had already deviated away from her projected growth pattern during the first few months of life while she was still being exclusively breast-fed, as indicated by the fact that she had crossed several percentiles during her first seven months of life. This suggests that her avid appetite may have been unfolding from birth. If this is true, it highlights the importance of monitoring an infant's appetite from the beginning of life to avoid accelerated early growth. However, it is interesting that KB was not overly concerned during this period, although as she highlighted, it is difficult to ascertain with any level of accuracy the amount of milk that a breast-fed baby is consuming. It may also be the case that health care professionals (such as health visitors) tend to be more concerned about growth-faltering than rapidly growing infants during this early period, so would not highlight her excessive growth to the parents as a cause for concern. S is also her first child so it may not have been clear to her mother that her milk-feeding behaviour was unusual. Another possibility is that S's responsiveness to milk was less aggressive than her appetite for solid food, given the monotony of a milk diet in comparison to the variety and palatability of solid foods.

KB clearly felt that her daughter's relationship with food reflected her biological father's appetitive traits, and pointed out that other members of his family were also very overweight, believing that her daughter's characteristics were inherited from his side of the family. This is supported by the findings in this thesis that individual differences in appetitive traits (such as food responsiveness and satiety sensitivity) are manifested very early in life, reflecting genetic differences. S's eating behaviour bears closer resemblance to her father's genetic makeup than her mother's, and it seems unlikely that at this early age her eating behaviour would be learned rather than innate given that her father has never been her primary care giver. It remains to be determined if S's appetite is being caused by any known genetic mutation. However, it is unlikely that one will be identified

given that only 5-7% of cases of early onset severe obesity have a known genetic cause (Farooqi & O'Rahilly, 2006). Her obesity was not found to be caused by an endocrine disorder either, nor was it a comorbid feature of other developmental anomalies known to be caused by major genetic mutations such as Prader Willi Syndrome, Bardet-Biedl Syndrome, and others (Farooqi & O'Rahilly, 2005).

It is possible that S has an unusual combination of common molecular variants that individually cause small differences in appetite, but the sum of which can promote an intense appetitive drive. A number of the 32 common genetic variants that have been found to relate to weight appear to influence energy balance centrally (Speliotes et al., 2010). Even though each individual variant contributes only a tiny amount to weight variation, collectively explaining only 1.45% of BMI variance, the difference in weight between individuals carrying $\geq$38 risk variants and those carrying $\leq$ 21 risk variants was 7-9 kilos for participants 160-180 cm tall. If another 284 loci of similar effects are identified, as predicted by the group, the weight difference between the individuals at highest and lowest risk will be far larger.

It is also possible that S has inherited one of the rare genetic mutations (or a collection of rare variants) known to influence weight. Her behaviour around food is typical of the appetitive phenotypes that characterise individuals with rare monogenic mutations known to cause early onset severe obesity, including mutations in the leptin gene (Farooqi et al., 1999; Farooqi et al., 2002; Montague et al., 1997), the leptin receptor gene (Clement et al., 1998), the POMC gene (Challis et al., 2002; Echwald et al., 1999; Krude et al., 1998; Miraglia del et al., 2001), and the MC4R gene (Dubern et al., 2008; Farooqi et al., 2000; Farooqi et al., 2003; Hinney et al., 1999; Kobayashi et al., 2002; Mergen et al., 2001; Vaisse et al., 2000; Yeo et al., 2003) to name just a few. The primary feature of all known monogenic obesity mutations is an insatiable appetite characterised by intense hyperphagia and no apparent satiety sensitivity as a result of major disruption to specific homeostatic mechanisms described in Chapter 2 (O'Rahilly et al., 2003).

Many of the distinctive aspects of these known mutations fit in well with observations that have been made about S. Clinical phenotypes of individuals with mutations to the leptin

receptor gene are: (1) deviation from predicted growth percentiles during the first 12 months of life; (2) active food seeking behaviour in childhood (which continues on into adulthood); (3) aggressive behaviour if food is denied, and; (4) in ad libitum test meals affected subjects consume about three times the amount of food eaten by controls (Farooqi et al., 2007b); these are all features of S's profile. Also of interest is the report that individuals with congenital leptin deficiency prior to leptin treatment gave high liking ratings to all foods (regardless of palatability and energy density) not preferring one type of food over another, but liking ratings were reduced substantially for all foods following leptin replacement therapy, and this was accompanied by reduced activation of the mesolimbic neural circuits governing reward in the fed state compared to the fasted state (in keeping with normal-weight controls), suggesting that leptin modulates wanting of food as well as satiety (Farooqi et al., 2007a). It is worthy of comment that S's mother, her caregivers, the food diary and the ad libitum meal, all attest to the fact that S will eat anything, as long as she is given food, which appears to be a characteristic feature of disruption to the neural control of appetite.

The amount of food consumed by S during the ad libitum meal was extreme at 1107 kcal, taking in 89% of her total daily energy requirement after having eaten her normal breakfast in the morning. However, this level of intake is in line with that of older children with known genetic mutations – for example, a nine-year old girl with congenital leptin deficiency weighing 94.4 kgs consumed 1600 calories during a test meal prior to leptin treatment, representing about 87% of her estimated daily energy requirement (1840 kcal) based on her basal metabolic rate (Farooqi et al., 1999), although it was a more reasonable proportion of her daily needs if calculated as a ratio of her body weight (28% of an estimated 5758 kcal) (Department of Health, 1991). Very little work has been done to characterise the normal range of energy intake in an ad libitum meal by children this young, making it difficult to draw conclusions about the magnitude of S's intake in comparison. However a study of 4 to 6 year old children (n=16) at day care centres measured the total energy intake each day, as well as that for each meal, over 5 to 7 days using food weighing (Mrdjenovic & Levitsky, 2005). At each meal except the evening meal, the amount consumed was less than the amount offered for each child, and no child consumed more than 700 kcal for any one meal recorded over the study period; in particular, for lunch (the equivalent meal to the one observed for S) the mean kcal

consumption was 274 (sd=142), and the maximum was 600 kcal despite more food being provided than was consumed for all children. S consumed almost double the amount of the maximum kcal consumption reported for an equivalent meal in this study of older children.

There was a considerable mismatch between the dietary data recorded in the diary and the amount consumed by S during the ad libitum meal, and her average daily intake was also somewhat lower than expected given her body size. However, KB made it clear that she has never allowed S to eat as much food as she would like, so it is not surprising that her normal intake was lower than her ad libitum intake. However, the intake still seems lower than would be expected for her body weight. It may have been the case that S's mother wanted to demonstrate her efforts to us in her restriction of S's daily food intake and to show that she was providing her with a healthy, balanced diet. It may also be true that for a considerable proportion of the time S is only allowed to consume an average amount of food for her age (slightly less than her daily requirements), but that caregivers give in to her from time to time allowing her to consume far more than she would normally eat in an average day accounting for the energy gap; but this did not appear to happen during the period of recording. However, this would be at odds with the comments of the nursery staff in the letter sent to KB that stated that S consumes a larger portion of food than other children her age. Birch and colleagues (1991) described the variability of ad libitum energy intake from 2700 calories offered per day over a six day period in 15 preschool children aged 26 to 62 months, during which intake was estimated under highly controlled conditions by weighing the foods before and after each eating occasion. Average intake each day ranged from 1100 to 1800 kilocalories, and the coefficient of variation for daily intake was small (10.4%) indicating considerable stability in intake within individual children. According to this study, S's day-to-day intake is well within the normal range and demonstrates that the efforts made by those caring for her are currently effective in restricting her intake to help avoid very rapid and excessive weight gain if the diary information reflects her actual intake.

Farooqi and O'Rahilly (2006) have suggested that any child less than 5 years old who is demonstrating extreme hyperphagia and severe obesity, and who has a family history of early-onset obesity, should be screened for genetic mutations underlying their

characteristics. The Genetics of Obesity Study set up in 1997 by their research team at the University of Cambridge has recruited over 2200 individuals (mainly children) globally who fit this description in order to identify more rare variants responsible for early onset obesity – only 7% of the sample currently have known mutations, highlighting that much more work is needed in this area in addition to the research challenge of identifying common variants with small effects (Farooqi & O'Rahilly, 2006). Given S's characteristics it was surprising that the healthcare professionals at Great Ormond Street Hospital were reluctant to take her into their care at first, protesting that she was too young and that her obesity was not yet severe enough. The problems S's mother has encountered in obtaining appropriate help for S highlights the difficulties faced by parents of overweight children generally – S's GP contacted me recently and commented that he was not aware of any help that is currently available to manage avid eating behaviour in children. He therefore contacted our research department. Given the fact that excessive weight gained early in life proves very difficult to reverse, it is important to intervene as early as possible to try to avoid the development of obesity in the formative years. For S, it could have been useful to have slowed her growth rate even in the first few months of life during the period of exclusive breast-feeding. The Baby Eating Behaviour Questionnaire may provide a useful tool for identifying at risk infants from the very beginning of life so that their developing appetitive characteristics might be monitored by parents and caregivers before weight problems develop.

Lastly, and in relation to the aforementioned point, the case of S has highlighted the need for well-designed intervention strategies that are successful in attenuating these traits, or at least managing them. I found I had little practicable advice to offer S's GP when he contacted me seeking help, over and above the strategies that S's mother is already implementing; her own methods reflect much of the work that has already been done, particularly her focus on slowing down the rate with which S consumes her food. An early intervention study by Epstein and colleagues (1976) succeeded in slowing down the eating speed (bites per 10 seconds) of obese and non-obese 7 year old children over a 6 month period by encouraging them to put their knife and fork down after each bite, and as a result the children consumed significantly less food. However, another more recent study that attempted to retrain the eating speed of older children using a mandometer was not successful in slowing eating rate (Ford et al., 2010).

A couple of recent intervention studies indicate that it may be possible to modify other 'obesogenic' eating behaviours in children. An Iranian study used cognitive restructuring to alter 11-15 year old obese children's relationships with food, and taught them strategies to manage their responsiveness to food stimuli; this was effective in reducing their scores on 'emotional eating', 'external eating' and 'restrained eating' (measured using the DEBQ) compared with the control group (Sabet et al., 2009). While such a cognitive intervention may not be suitable for very young children such as S, Johnson (2000) published a promising study showing that it is possible to improve compensation ability (measured using a preload paradigm) in preschool children aged 4 to 5 years. The intervention that took place in weekly sessions over 6 weeks involved using dolls and videotapes to teach the children interactively about recognising and attending to internal satiety sensations before and after snack food. However, the children in this study included a range of weights, and it may be the case that children with highly upregulated appetites such as S do not benefit as much from this type of intervention. Additional research is needed in this area to measure intervention success with children who are demonstrating extreme eating behaviour.

In conclusion, S's eating behaviour illustrated how a highly upregulated appetite manifests itself in very early life. Her case also highlights the need for early interventions both to help parents to recognise abnormally intense appetitive traits as early as possible, and to develop methods to attenuate their expression. The description of S makes it clear that eating behaviour does not simply reflect an individual's will power with regard to food self-regulation; rather, for some individuals the drive to eat is as powerful as other biologically controlled phenomena such as sleeping and waking.

CHAPTER 12. CONCLUDING DISCUSSION

12.1. Summary of thesis findings

The overall aim of this thesis was to test one of the assumptions of the behavioural susceptibility model of weight, namely that inherited individual differences in appetite are already present in infancy, and that shared genetic effects are contributing to phenotypic associations with weight from the beginning of life. In order to achieve this goal, I completed a number of studies that individually helped to piece together each part of the picture, and collectively provide a good case for a behavioural susceptibility model of weight.

The development of the Baby Eating Behaviour Questionnaire (Study 1, Chapter 6), based upon the CEBQ (Wardle et al., 2001b), provides the first psychometric parent-report measure of appetite for infants who are still exclusively fed milk. Four underlying dimensions of appetite were identified, akin to those observed in children: 'enjoyment of food', 'food responsiveness', 'slowness in eating', 'satiety responsiveness', as well as a single item of general appetite avidity ('appetite size') that correlated with all four traits. The BEBQ was able to distinguish infants with different characteristics (such as premature infants, or those with feeding problems), and the appetitive traits showed many of the same relationships as those observed in the equivalent CEBQ characteristics in children (e.g. Carnell et al., 2008; Sleddens et al., 2008; Viana et al., 2008; Wardle et al., 2001b). The results showed that appetite can be measured from early in life, and that there are individual differences in appetitive traits even before any solid food has been introduced.

Individual differences in all of the appetitive traits of the BEBQ were associated with weight variability at three months, collectively explaining 10% of the variance, and with change in weight from birth to three months, collectively explaining 4% of the variance (Study 2, Chapter 7). The associations were in the same directions observed in children using the CEBQ (Carnell et al., 2008; Cunha et al., 2010; Gregory et al., 2010; Jahnke & Warschburger, 2008; Joyce & Zimmer-Gembeck, 2009; Parkinson et al., 2010; Sleddens et al., 2008; Viana et al., 2008; Webber et al., 2009) and supported the few behavioural studies in infants (Agras et al., 1990; Stunkard et al., 2004) – a more avid feeding style

predicted greater adiposity. This finding raises the possibility that individual differences in appetite that are present from birth are contributing to the development of weight over the first few months of life.

For the first time, the role of genetic differences in explaining appetite variability in the first few months of life, while infants are still only fed milk, was assessed (Study 3, Chapter 8). All the appetitive characteristics were moderately to highly heritable: 'enjoyment of food', 83%; 'food responsiveness', 59%; 'slowness in eating', 84%; 'satiety responsiveness', 72%; 'appetite size', 77%. These estimates were in keeping with those observed in children (Carnell et al., 2008; Llewellyn et al., 2008), and suggest that the individual differences observed in appetite reflect inherited genetic variation. Some of the genetic influences involved in the regulation of the different appetitive traits were shared. A common genetic pathway underlying 'enjoyment of food', 'slowness in eating' and 'satiety responsiveness' was identified that explained 78% of the covariation between them, although 'food responsiveness' appeared to be genetically distinct (Study 4, Chapter 9).

Chapter 10 used a multivariate genetic analysis to answer the key question in this thesis – do appetite and weight share a common genetic pathway from the beginning of life? A quarter to a third of the genetic influences on 'slowness in eating', 'satiety responsiveness' and 'appetite size' also influence weight at 3 months, and shared genetic effects on both account for about half of the observed phenotypic associations between the appetitive characteristics and weight (Study 5, Chapter 10). Together with studies 1-4, this final analysis provided a very good case for proposing that a behavioural susceptibility model of weight explains the seeming paradox of both genetic and environmental determination of weight, and suggests that the interplay between genes and the environment in the development of adiposity begins at the beginning of life. The only other study to have looked at this issue used a sample of adults; although the number of common genetic effects were comparable, a considerably higher proportion of appetite and weight covariation was explained by shared genetic influences (81% to 100%) (Keskitalo et al., 2008). This suggests that the role of common genetic effects that drive weight through appetite snowball over time, perhaps because the increased independence that comes with maturation means that individuals are more able to act in line with their genetically-determined appetitive dispositions, and thereby allow the genes that influence appetite to influence weight more freely.

The final chapter of this thesis (Study 6, Chapter 11) used an in-depth exploration of a single case of an 18-month old infant (S) with extreme appetitive avidity to describe in detail the eating behaviours that she was presenting, to demonstrate that the traits were manifested during the first few months of life, to show that she was already obese by 7 months, and to discuss the familial basis for her appetite and weight. S's eating styles bear close resemblance to those seen in individuals with obesity-causing major genetic mutations (Farooqi et al., 1999; Farooqi et al., 2007b; Farooqi et al., 2007a; Stunkard et al., 2004) As a result of her hyperphagia, her parents were forced to exert drastic control measures to avoid severe overeating, and prevent more excessive weight gain in the future.

The findings in this thesis have a number of practical and theoretical implications, and pave the way for future work. These are discussed below in section 12.2. The studies in this thesis have a number of strengths and advantages over past research, but there are also some limitations which must also be acknowledged; the strengths and weaknesses of the thesis are outlined in section 12.3.

12.2. Implications for theory, practice and future research

12.2.1. Intervention work

Finding such high heritability estimates for many of these appetitive characteristics has practical implications for intervention work. A common misconception about genetic influences on traits such as these is that environmental modification is not possible. In reality, control of environmental conditions could alter expression of genetic phenotypes (e.g. tendency to overeat may depend on exposure to highly palatable food). As these traits have a high level of genetic influence, interventions to modify their expression may be most effective if targeted at the individual level, selecting those infants who are demonstrating upregulated appetitive responses. A further consideration is the importance of identifying infants in the earliest phase of life so that attempts can be made to attenuate the expression of these traits before excess weight gain has occurred and the full genetic

effect has become difficult to reverse, such as with the case of S. The BEBQ may provide a useful tool for this task.

The case of S also highlights the need for well-designed and properly tested interventions that are successful at ameliorating the expression of these traits in early life, and the need to check the efficacy of the techniques at different levels of phenotypic intensity. Currently very little intervention research has been done in the area of eating behaviour in children, and even less during infancy. Practicable options may include using a slower flowing teat if bottle-feeding to reduce feeding rate, feeding limited amounts of milk to infants who are highly food responsive in spite of protests for additional feeds or quantities, and lowering the energy density of formula milk by changing the ratio of powder to water. The latter suggestion is currently being investigated in a randomised controlled trial at Cambridge University, and the effects on the development of appetite are being evaluated using the BEBQ.

12.2.2. Identifying molecular genetic variants and neurological and physiological endophenotypes

Finding such high genetic influence on appetite, and genetic commonality between appetite and weight, makes it worthwhile looking for molecular genetic variants influencing both, especially in the light of the recent GIANT consortium meta-analysis of GWAS showing that a sizeable proportion of the 32 SNPs related to adiposity are likely to be operating through central appetitive mechanisms (Speliotes et al., 2010). Although Gemini is not the appropriate sample for conducting GWAS to find novel SNPs relating to appetite and weight due to the limited size, the twin design provides a powerful method for replicating associations with weight, and exploring relationships between candidate genes and appetitive characteristics (e.g. the variants highly expressed in the hypothalamus or gut), while controlling for shared environmental influences, ethnicity, and other genetic influences that create additional ‘noise’ in standard population-based cohorts. For example, DZ twin pairs discordant for a genetic variant of interest (e.g. *FTO*) can be used to assess appetitive differences (or weight differences), controlling for other factors shared by a twin pair, including ~50% of other genetic effects, arguably providing a ‘cleaner’ picture of genetic associations to be determined (Eaves & Meyer, 1994); genetic theory suggests that there is a 50% chance that DZs will be discordant for each genotype. In

addition, genetic models can be extended to include measured genetic polymorphisms so that the amount of genetic variance accounted for by the gene of interest can be determined, as well as the residual unexplained genetic variance (Martin et al., 1997).

Work has already begun to select and measure variants of interest for Gemini. I conducted a review of the literature (primarily using online genetic epidemiology databases such as the HuGE Navigator, Version 2.0) to identify SNPs that have been related to appetitive traits or eating behaviours in all age groups, and those related to weight and growth. Genetic variants whose association with appetite or weight had been replicated at least once were included in a final shortlist of SNPs that was sent to Ruth Loos at the University of Cambridge, who is a member of the GIANT consortium and an expert in this area. Following her comments the final list included 28 SNPs. The Gemini infants' DNA is currently being genotyped at a laboratory at the Institute of Psychiatry, and we hope to receive the results in a few months. We will be able to use DZs discordant for genotype to look at the involvement of these SNPs in the phenotypes of interest; as far as I am aware this has not yet been done in appetite or weight research.

There is a considerable mismatch between the high heritability estimates for appetite and weight (at least at older ages) and the very small effect sizes of the variants identified so far, making it likely that there are hundreds of genes involved. For example, the 32 SNPs identified by Speliotes and colleagues' (2010) still only explain 1.45% of the variance in BMI, a far cry from the magnitude of heritability estimates from twin studies. If they are right in predicting that there are another 284 loci with comparable effect sizes the total variance explained increases only to 4.5%, making the gap between heritability and molecular genetic explanations considerable. The 'missing heritability' may be partly explained by non-additive genetic effects such as epistatsis (gene-gene interactions). This can be taken into account in twin designs but has to be measured specifically in molecular genetic studies – some researchers in the field of molecular genetics have found epistasis to be the rule rather than the exception (Carlborg & Haley, 2004), and some twin studies have estimated that up to half of the genetic effects are non-additive (Maes et al., 1997). Epistasis complicates relationships and it will be fruitful to account for it in Gemini when determining the influence of measured SNPs on appetite and weight. It may also be the case that many of the variants that influence an individual's weight are rare, making it unlikely that they will be identified through GWAS.

Chapter 9 showed that there is considerable genetic overlap among 'slowness in eating', 'satiety responsiveness' and 'enjoyment of food', suggesting that many of the genes that underlie these traits have pleiotropic effects. An alternative explanation is that each of those appetite scales tap aspects of the other characteristics as well as the trait of interest, so the genetic overlap comes from imprecise measurement rather than pleitropy (e.g. the genetic correlations may have arisen from the 'slowness in eating' scale also tapping aspects of the trait indexed by the 'satiety responsiveness' scale). Gene expression mapping is a fairly recently developed method that allows the expression of a gene to be mapped throughout the brain using RNA (Cheung & Spielman, 2009; Greenberg, 2001; Petretto et al., 2010; Yamasaki et al., 2005). Genetical genomics uses this method to study variation in gene expression in relation to genetic variation within given loci (Cheung & Spielman, 2009). If a particular variant, such as *FTO*, is related to multiple appetitive characteristics, and different *FTO* variants show differential expression across multiple brain regions, there are grounds for hypothesizing pleitropic effects rather than measurement overlap. So far, findings have indicated that most genes tend to be expressed across many brain regions rather than locally, making it probable that variants involved in the central regulation of appetite influence more than one process (Plomin et al., 2007), and making it possible that there is interplay between homeostatic and hedonic processes at the level of gene expression.

Identifying molecular genetic variants involved in appetite avidity gives rise to another avenue of research – elucidating the central (e.g. neuropeptides) and peripheral (e.g. gut hormones) endophenotypes that mediate the relationship between the SNPs of interest and the measured eating behaviours or appetitive characteristics (e.g. which neurotransmitters mediate the relationship between FTO and satiety sensitivity?). Delineating intermediate pathways is more straightforward using animals because genotype and gene expression can be manipulated (e.g. Church et al., 2010), environmental exposures may be closely controlled, and post-mortem examinations of cell or tissue function can be undertaken. But conclusions drawn from animal research are not easily generalised to humans. Research in human subjects is more limited, and even more so in children and infants. However the advent of functional neuroimaging techniques such as functional magnetic resonance imaging (fMRI) has opened up opportunities to 'map' appetitive characteristics on to neural processes and molecular genetic variants such as

FTO. For example, a sample of individuals who vary in their *FTO* genotype status could be asked to eat to satiety in an ad libitum meal and then scanned while being exposed to food cues; finding differences in reward processes that are predicted by genotype would suggest that the neural processes are linked to differential *FTO* expression. If blood samples are taken as well, peripheral chemical signals can also be taken into account. An innovative study in this area used fMRI to demonstrate that striatal activation in response to food intake was not only related to prospective weight gain, but that this association was mediated by the presence of the A1 allele of the Taq1A polymorphism associated with dopamine D2 receptor gene binding in the striatum and attenuated striatal dopamine signaling (Stice et al., 2008).

Neuroimaging in very young children is challenging, not only because of anatomical differences, but also because of procedural requirements such as no movement of the head during scanning, and the exposure to high magnetic fields which raises safety issues (Davidson et al., 2003). However, a good alternative may be a novel method called Near Infrared Spectroscopy (NIRS) whereby it is now possible to study the infant brain without the restrictions of fMRI (Aslin & Mehler, 2005), and this method has been used to identify the neural activity underlying language ability, visual capacity and language (Baird et al., 2002; Pena et al., 2003; Taga et al., 2003). As far as I am aware, no study has used this method to study the neural basis of appetitive traits in infancy, and most research in this area so far has been at the normative level, rather than individual differences. The Centre for Developmental Cognitive Neuroscience at UCL is leading the way in understanding how NIRS might be used to study the neural basis of infant characteristics, making it possible to include this element of investigation into the Gemini study to better understand the brain pathways underlying these traits in early life. This future work will require collaborative relationships with neuroscientists and endocrinologists, which I am in the process of seeking out.

12.2.3. Identifying environmental influences

The search for environmental influences that shape appetite (and weight) is potentially more challenging than identifying genes. We know how to measure genes and methods for understanding gene function are advanced. Chapter 2 outlined the biological basis for appetite, showing that at the most basic level eating behaviour is governed by processes

that are regulated and directed by the brain, including cognitive processes or hedonic and homeostatic mechanisms. Presently, we know virtually nothing about how environmental factors influence the brain (either temporarily or permanently) to direct eating behaviour, or how they cause individual differences in appetite to develop (Plomin et al., 2008). Twin studies do not provide any insight into the nature of the shared and unique environmental influences themselves, and aspects of the environment that influence appetite may be shared for some infants and not by others, such as bottle-feeding or breast-feeding infants, and parental feeding styles.

A powerful design to identify influences of the unique child environment is to study MZ differences – if differences between MZs on any given trait (or set of traits) predict differences on another trait (or set of traits), they must, of necessity, be unshared between the two twins (i.e. due to unique environmental exposures). A possible design to assess which environmental factors influence multiple appetitive traits (indicated by the modest unique environmental correlations between 'enjoyment of food', 'slowness in eating' and 'satiety responsiveness') would be to associate differences in the latent component scores (which capture the covariance between the traits) with differences in aspects of the unshared environment such as differential parental feeding styles. To identify important drivers of both appetite and weight, MZs who are discordant for both can be used. Just as it is possible to include measured genotype information into multivariate models, so it is also possible to include measures of unique environmental influences (any variable for which each individual twin has their own score). Shared environmental effects may be studied by comparing twins who are both concordantly high on appetite (or appetite and weight) with those who are concordantly low. It is not possible to include shared environmental measures in multivariate models, but the twin sample can be divided into sub-groups by the variable of interest (e.g. a heterogeneity model including bottle-fed and breast-fed infants), and parameter estimates compared. Huge efforts have been made in Gemini to measure the environment in general, and wherever possible to take measures for each individual child, making it possible to explore a host of potential shared and non-shared environmental shapers of early life appetite and weight.

12.2.4. Linking milk-feeding behaviour with eating behaviour

A key question is whether appetite for milk is the same as appetite for food – i.e. are the characteristics captured by the BEBQ the predecessors of the corresponding CEBQ traits on which they were based? We have measured appetite for food at 15 months in Gemini using the CEBQ so it will be possible to answer this question by evaluating the magnitude of the phenotypic correlations between the corresponding scales of the BEBQ and CEBQ. As well as determining the phenotypic stability of the traits over time, it will be of interest to explore how genes and the environment contribute to trait stability and change. This can be assessed using a longitudinal Cholesky Decomposition model. The proportion of genetic variance in the traits at 15 months that is accounted for by the genetic influence during the milk-feeding phase can be estimated (the genetic correlation), as well as the genetic influence that is unique to 15-months (i.e. new and unshared genetic effects). Additionally, the role that continuing genetic effects play in trait stability (the longitudinal phenotypic association) can be quantified by calculating the longitudinal bivariate heritability (the proportion of the longitudinal association that is explained by continuing genetic effects).

12.3. Strengths and weaknesses

12.3.1. Strengths

The studies in this thesis provide a number of advantages over previous research. Study 1 described the development of the first psychometric measure of infant appetite. The scales bear close resemblance to those of the CEBQ, on which they were based, the Cronbach's alphas were high suggesting that the scales are reliable, and the underlying structure was reproduced in 10 reasonably sized sub-groups of infants, highlighting the robustness of the findings. Since the development of this questionnaire, many researchers have contacted me to ask if they can use the BEBQ for their research, commenting that this is a much-needed instrument. It has been translated into a number of different languages which will allow the component structure to be replicated in other populations.

Gemini is a very large, population-based cohort of infants, allowing for small associations with weight to be estimated reliably, and for heritability to be established with precision. 96.4% of the weight data at 3 months were measured by healthcare professionals, providing a significant advantage over parent-report measures of weight, and a number of potential covariates between appetite and weight were measured and accounted for in analyses.

The studies in this thesis used a variety of methods to explore the relationship between appetite and weight in infancy in order to create a detailed picture of the dynamics between them – the magnitudes of the associations were estimated, the interplay between genetic and environmental factors in driving the relationships was determined, and an in-depth exploration of an extreme case was undertaken. The findings concurred that appetite and weight share a close relationship for which there is a common genetic basis. However, there are also some limitations to these studies, which are discussed below in section 12.3.2.

12.3.2. Weaknesses

12.3.2.1 BEBQ needs to be validated

Firstly, and most importantly, the BEBQ needs to be validated using behavioural measures of appetite to check that parental ratings truly reflect individual differences in the traits the scales are intended to measure, and not parental biases such as assigning a greater appetite avidity to infants who are bigger in order to explain their body size. In addition, the findings need to be replicated in singletons, and using concurrent measures of appetite rather than retrospective; the temporal stability of the BEBQ also needs to be tested.

I have already begun the work for this by single-handedly designing and setting up the Baby Eating and Baby Appetite Study (BABES) for which ethical approval has been given, and recruitment, pilot work and data collection proper has started. BABES is an intensive study of appetite and weight in exclusively milk-fed infants less than 4 months of age, that was devised following an in-depth review of the literature on behavioural measurement of

infant milk feeding and appetite. A 3-day milk-feeding diary has been designed, along with two infant feeding tasks which take place in the mother's home and are videotaped by the researcher so that the sucking behaviour can be coded subsequently using Noldus software. Firstly, the infant is filmed during an ad libitum milk feed for which he or she is given an unlimited amount of milk to consume, and the bottle weighed every two minutes to ascertain the total amount consumed, the rate of consumption, and feeding deceleration; 30 minutes later the infant is offered another feed under conditions of assumed satiety (similar to 'eating in the absence of hunger') and the amount consumed is weighed. During the home visit, the mother also completes a concurrent version of the BEBQ, and a second copy is left for her to complete two weeks later. The information obtained from the feeding tasks and the diary will be used to validate the BEBQ scales, test-retest reliability of the BEBQ over a two week interval will indicate temporal stability, the singletons will allow for replication of the underlying structure of the BEBQ in a non-twin sample, and finding the same appetite dimensions for a concurrent version of the BEBQ will provide credibility for the retrospective version[88].

12.3.2.2. Generalisation to singletons and other populations

Heritability estimates are sample-specific and so findings cannot be generalised to other populations. Gemini is largely White-British so it would be useful to replicate these findings in other ethnic groups. There were insufficient numbers of infants from other well-defined ethnic groups in the current study to look at parameter differences for this characteristic, although neither appetite nor weight differed significantly between White-British and non White-British groups.

Because twins are born smaller and earlier than singletons (Buckler & Green, 2004; Grumbach et al., 1986), genetic influences on appetite may be different. Family studies tend to report slightly higher heritability estimates for weight during the first few months of life than twin studies, probably because of the growth restriction of twins in utero (Bouchard, 2009). We have started to collect BEBQ data on younger siblings of the Gemini twins, and will collect weight data; this can be incorporated in to the genetic

[88] The materials for the Baby Appetite and Baby Eating Study are shown in Appendix 9. The protocol for the home visits is shown in Appendix 9.1, the feeding tasks data collection sheet is shown in Appendix 9.2, and the milk feeding diary is shown in Appendix 9.3.

models to explore heritability estimates following inclusion of singleton siblings. However, other studies that have incorporated a multitude of other relatives (e.g. the Virginia 30,000 study with 10,000 twins and 20,000 other relatives including parents, siblings, spouses and off-spring of the twins) have found that the genetic and environmental estimates for a whole range of traits including adiposity are virtually identical to the twin estimates alone (Maes et al., 1997; Truett et al., 1994).

12.3.2.3. Cross-sectional design

All of the studies in the thesis used cross-sectional data which means it is not possible to draw conclusions about the direction of causation. Weight and appetite could be associated because greater adiposity causes greater appetite avidity, and finding common genetic pathways underlying the appetitive traits and weight could reflect the fact that weight is heritable and individual differences in weight cause corresponding variation in appetite. However, I believe that this is unlikely on three counts: (1) a number of longitudinal studies with children and infants have found that eating or feeding behaviour predicts weight gain prospectively, even after adjustment for baseline adiposity level (e.g. Agras et al., 1990; Hill et al., 2009a; Parkinson et al., 2010; Rodin & Slochower, 1976; Stunkard et al., 2004); (2); differences in these appetitive traits are able to distinguish infants and children who are genetically predisposed to obesity on the basis of their parents' weight from those who are most likely to remain lean, before weight differences have appeared (e.g. Millstein, 1980; Stunkard et al., 2004) (3) a sizeable proportion of the molecular genetic variants that relate to weight, identified through the most recent meta-analysis of the GIANT consortium, are most highly expressed in areas of the brain involved in the homeostatic regulation of appetite (Speliotes et al., 2010). This suggests that the most likely pathway underlying the observations here is:

genes ⟶ appetite ⟶ weight.

Because Gemini is a large prospective study it will be possible to explore the prospective relationship between appetite and weight. Because we have measured both appetite and weight at more than one time-point, a possible method would be to ascertain if the correlation between appetite at baseline and weight at 15 months is as strong as the correlation between weight at 3 months and appetite at 15 months. A sophisticated

method for partitioning out confounding effects of weight on appetite or appetite on weight at each time point would be to use a genetically sensitive prospective design whereby the genetic contribution to the longitudinal association between appetite at 3 months and weight at 15 months is determined, after adjusting for weight at 3 months and appetite at 15 months. This is my next task.

12.3.2.4. Gene-environment correlations

Heritability estimates include the sum of the effects of genes, but also include gene-environment correlations (explained in Chapter 4) – i.e. the genetic variance also subsumes variance due to genetically-driven environmental exposure. It is conceivable that in infancy the heritability estimates for appetite may partly reflect 'evocative' gene-environment correlations – the elicitation of environmental responses by genetically influenced behaviours (Bergen et al., 2007; Plomin et al., 2008; Scarr & McCartney, 1983). During this early period of life mothers are highly responsive to their infants' feeding needs as many of the nurturing duties centre around feeding. It is therefore possible that infants who are more demanding with regard to being fed (i.e. score highly on 'food responsiveness') are fed more often than less food responsive infants, and infants who score low on 'satiety responsiveness' are fed larger quantities of milk than those who appear to get full easily. This process can reinforce the trait by causing the genetic effect on the characteristic to snowball, increasing the phenotypic variance in any population, and strengthening similarities between individuals who are more closely genetically related (i.e. MZs versus DZs) which in turn increases measured heritability (Plomin et al., 2008).

For example, mothers of MZ twins who are concordantly high on the genetically determined appetitive traits 'food responsiveness' and enjoyment of food' may be more likely to respond to them with similar feeding methods (such as topping up breast feeds with a bottle in order to meet their needs). This may serve to intensify these traits for both MZ twins, and make them more similar than DZ twins for whom less genetic resemblance results in less similar phenotypic expression, and therefore more disparity in feeding methods. There was a suggestion in Gemini that this type of gene-environment correlation may be present because a slightly (but significantly) higher percentage of MZ twins were fed using the same method (97.4%) than DZ twins (92.2%), although this may reflect the slightly higher incidence of feeding problems in MZs as well as the greater concordance

for feeding problems in MZs. Gene-environment correlations do not constitute a violation of the equal environments assumption because the differential environmental treatment is driven by genetic differences (Plomin et al., 2008).

12.3.2.5. Equal environments for MZs and DZs and parental rating biases

A slightly greater incidence of feeding problems and concordance for feeding problems in MZs could reasonably constitute a violation of the equal environments assumption for the estimation of appetite heritability. However, heritability was estimated with and without all problem-feeders to check for this confounder and the estimates, although slightly higher, were virtually the same, suggesting that this effect is minimal. There is also the possibility that sharing a placenta makes twins more (or less) similar due to differential nutrient transfer (Loos et al., 2005a; van Baal & Boomsma, 1998). 70% to 75% of MZs share a placenta (i.e. are 'monochorionic') while all DZ twins have separate placentas[89] (i.e. are 'dichorionic'). Obtaining accurate information on chorionicity is difficult and requires careful analysis and documentation from healthcare professionals involved in the delivery of the twins. We did not have appropriate information on this issue in Gemini so were unable to test for differences.

However, we did have information on whether the parents thought their twins were MZ or DZ and I was able to use this information to test if parents who misclassified their MZs as DZs or their DZs as MZs (according to our zygosity questionnaire) rated them as more or less similar than parents who correctly classified their twins. The twin correlations were virtually the same for all the appetite scales, and estimates of heritability were very similar using different subgroups, and in every case the 95% confidence intervals overlapped. In addition, the tests for parental rating biases in the structural equation models did not find any variance differences between MZs and DZs, nor were the MZ correlations 'too high' nor the DZ correlations 'too low' (the hallmarks of rating biases) (Saudino et al., 2000). Together, these findings suggest that the heritability estimates are not influenced by parental biases (i.e. parents of MZs scoring them more similarly than they actually are because they believe them to be identical) but reflect true genetic differences. A host of other studies that have tested this assumption using mistaken zygosity have also

[89] In extremely rare cases the two placentas fuse for DZs, giving the impression that they are monochorionic (Redline, 2003; Souter et al., 2003).

concluded that twin similarity is not influenced by twin labeling (Gunderson et al., 2006; Kendler et al., 1993; Scarr & Carter-Saltzman, 1979).

12.3.2.6. Assortative mating

There was a small indication of assortative mating (or a shared environment effect) for adiposity among the Gemini parents who were correlated for both weight and BMI (0.23), in line with other studies (Allison et al., 1996; Jacobson et al., 2007; Mascie-Taylor, 1987; Silventoinen et al., 2003; Speakman et al., 2007; Tambs et al., 1991). Assortative mating would serve to lower heritability estimates for weight slightly as DZs would be share slightly more than 50% of their weight-related genes, inflating their correlation. If weight and appetite share a common genetic pathway, assortative mating on weight might also influence appetite slightly, although given the small correlation between parents and the modest phenotypic association between appetite and weight in infants this effect is likely to be minimal.

12.4. Conclusion

The overall aim of this thesis was to test one of the assumptions of the behavioural susceptibility model of weight – namely, that inherited individual differences in appetitive traits are already present in infancy, and are contributing to genetically-determined weight variability from very early on in life. This thesis provided four pieces of evidence that provide support for a behavioural susceptibility model of weight in infancy. Firstly, Study 1 established that individual differences in appetitive traits ('enjoyment of food', 'food responsiveness', 'satiety responsiveness', 'slowness in eating' and 'appetite size') are present and measurable from the first three months of life, before any solid food has been introduced, suggesting that these traits are manifested very early on in the lifespan. Secondly, Study 2 showed that appetitive differences are associated with weight during this early period – infants with more avid appetites had greater adiposity at 3 months and gained more weight between birth and 3 months than infants with smaller overall appetites, raising the possibility that disparities in appetite are causing weight differences to emerge even in the period of exclusive milk-feeding. Thirdly, Studies 3 and 4

established that these appetitive traits have a heritable basis: Study 3 showed that genetic differences explain the majority of individual differences in all of these characteristics, with ‘enjoyment of food’ (83%), ‘food responsiveness’ (59%), ‘slowness in eating’ (84%), ‘satiety responsiveness’ (72%) and ‘appetite size’ (77%) showing strong genetic influence; Study 4 identified a common genetic pathway underlying ‘enjoyment of food’, ‘slowness in eating’ and ‘satiety responsiveness’ that accounted for the majority of the covariation between them (78%).

Study 5 provided direct evidence for the behavioural susceptibility model because for the first time, many of the genes underlying ‘slowness in eating’, ‘satiety responsiveness’ and ‘appetite size’ were also shown to influence weight at 3 months of age, with moderate genetic correlations between appetite and weight in the region of 0.22 to 0.37. Furthermore, common genes played an important role in driving the observed associations between appetite and weight, explaining nearly half (41% to 45%) of the phenotypic covariation. This finding demonstrates for the first time that the commonality observed between eating behaviour and weight is genetically determined from the first few months of life, and inherited appetitive differences may be contributing to the heritability of weight variation in infancy. Collectively, these findings are consistent with the idea that inherited susceptibility to the current obesogenic environment may make some infants more likely to overeat than others and therefore more at risk of excessive weight gain, from the beginning of life. Differences in appetite that reflect genetic variation may determine the likelihood that an infant (or child) either overeats or appropriately regulates their intake in relation to their energy needs when food (or milk) is freely available. This is a potential mechanism through which differential genetic susceptibility confers either an increased risk of obesity or the ability to effectively (and effortlessly) maintain a healthy weight.

It is not possible to draw firm conclusions about the cause of direction between appetite and weight because the findings were based on retrospective parent-report measures of appetite, and a cross-sectional design. However, confirmation of the heritability findings using misclassified (by parents) zygosity would suggest that parental response bias was minimal, if present at all. Validation of a concurrent version of the BEBQ using observed feeding behaviour, and replication of the findings using prospective analyses would greatly strengthen the behavioural susceptibility model. Nevertheless, the findings in this thesis are supported somewhat by recent discoveries from the field of molecular genetic research

which has identified a number of SNPs that are both robustly associated with weight, and highly expressed in areas of the brain involved in central energy balance, such as *FTO* (Speliotes et al., 2010).

The findings in this thesis have implications for the obesity epidemic in terms of both public health policy and clinical intervention. If the present findings are replicated, the increases in adiposity that have been demonstrated at the higher end of the distribution are partly accounted for by differential susceptibility to the contemporary food environment, with the individuals who are most genetically at risk gaining the most weight. This calls into question the notion of personal responsibility for obesity. It is a commonly held belief that an individual's weight status is a reflection of their own self-directed decisions and actions with regard to food choice and consumption. Likewise, a child's weight status is deemed to be the responsibility of the parents insofar as rearing strategies are assumed to be the most important determinants of child eating behaviour and ultimately weight. Rather, these findings suggest that some children (more often those of overweight parents) will find it much harder than others to regulate their food intake appropriately in the presence of food cues because biological processes that are ultimately governed by genes potently direct their eating behaviour; this makes the fight against overconsumption for some a constant battle in the current environment. This is highlighted by the case of S.

A corollary of this is that volition or self-will alone may not be sufficient for susceptible individuals to control their eating behavior, or that of their children, in the given environment. A more effective strategy might be tighter statutory regulation of the wider food environment at a national or international level to provide an environment that is less likely to elicit overconsumption in the first place. Put more frankly, the burden of responsibility should not lie entirely with the individual (or the parent), but partly with the state. National or international legislation governing food quality and availability would go a long way towards ameliorating problematic aspects of the current food environment and preventing overconsumption, especially if consequences were severe should organizations violate the stipulations unlawfully. Within the UK, the government could introduce and enforce a range of environmental changes such as removal of vending machines stocked with 'junk food' from schools and areas frequented by children, tighter control of food marketing to children, limitation of the number of fast food venues, and regulation of supermarket layouts (e.g. Gostin, 2007).

Along the same lines, clinical interventions may be most effective if focused on environmental modification, but targeted at the individual level (or the trait that is most prominent). For example, parents of children who are extremely food responsive may be able to attenuate the expression of this trait by limiting their exposure to palatable foods – options include limited feeds for milk-feeding infants, cooking smaller amounts of palatable foods for dinner for children who are weaned which would remove the temptation for the child to want 'seconds', and keeping problem foods out of sight, or better out of the home. The findings here also suggest that it may be prudent to consider intervening very early on in the lifespan, even during the milk-feeding phase, so that parents of infants who are showing unusually avid appetites and rapid growth are given appropriate advice to help them to minimise the amount of weight gained from overconsumption of milk, and later of food.

Heritable susceptibility may be more important for the development of obesity than previously believed. Certain individuals may be much more likely to gain excessive amounts of weight than others because their genetic information encourages overconsumption of palatable food. While a behavioural susceptibility model clearly postulates that some individuals are at greater risk of obesity than others, and may imply that those individuals should be the focus of interventions, it in no way rules out the need for public health measures. Given that adiposity varies greatly across a continuum representing a diverse range of susceptibility, modifications to the wider food environment would arguably benefit all given that increases in weight even within the normal range are associated with negative health outcomes such as increased cancer risk (Renehan et al., 2008). A greater understanding of gene-environment interactions in obesity is needed if interventions at either the individual level or that of the wider environment are likely to be effective.

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APPENDICES

Appendix 1. Tables summarising the literature on associations between appetitive traits and weight in children

Appendix 1.1. Measures of food responsiveness and weight

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight status[c]	CI/ Non-CI[d]			
Halford et al., 2004	42	9-11 yrs	M=18 F=24	UK	NW=28 OW=9 OB=5	Non-CI	SAE (watching food adverts) - lab	CS-CC	Significant effect of weight group for food advert condition (OW & OB consumed more than NW).
Halford et al., 2008	59	9-11 yrs	M=32 F=27	UK	NW=33 OW=15 OB=11	Non-CI	SAE (watching food adverts) - lab	CS-A CS-CC	Significant association in food advert condition (higher BMI & higher energy intake). Significant effect of weight group in food advert condition (NW consumed the least and OB consumed the most).
Halford et al., 2007b	93	5-7 yrs	M=39 F=54	UK	NW=65 OW=13 OB=15	Non-CI	SAE (watching food adverts) - lab	CS-A CS-CC	Significant association in food advert condition (higher BMI & higher energy intake). No significant weight group difference in food advert condition.
Jansen et al., 2003	31	8-12 yrs	M=17 F=14	Dutch	NW=15 OW=16	Non-CI	SAE (smelling food) - lab	CS-CC	Significant group difference (OW children had higher intake following exposure).
Millstein, 1980[a]	-	Infants	-	-	-	-	SAE (sweetened solution)	CS-CC(P)	Significant group difference infants of OW parents had higher intake scores).
Butte et al., 2007	798	4-19 yrs	M=410 F=388	US H	NW=385 OW=413	Non-CI	EAH-lab	L-A	No significant longitudinal association (EAH & weight gain over 1 year).
Cutting et al., 1999	75	3-6 yrs	M=40 F=35	US W=87% non-W=13%	-[NR]	Non-CI	EAH-lab	CS-A	Significant association (higher EAH & higher BMI) for girls. No significant association for boys.

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight status[c]	CI/ Non-CI[d]			
Faith et al., 2006	53	5 yrs	M=27 F=26	US	PNW=25 POW=28	Non-CI	EAH-lab	CS-CC(P)	Significant group difference for boys (boys with OW parents had higher scores). No significant group difference for girls.
Fisher & Birch, 2002	165-181	5 & 7 yrs	F	US W	NW= 75% (5 yrs) 77% (7 yrs) OW= 23% (5 yrs) 25% (7 yrs)	Non-CI	EAH-lab	CS-CC	Significant group difference both ages (OW girls had higher EAH scores at 5 & 7 yrs).
Fisher et al., 2007	725	5-18 yrs	M=369 F=356	US H	NW=326 OW=399	Non-CI	EAH-lab	CS-CC	Significant group difference (OW children had higher scores).
Frances & Birch, 2005	159	5,7,9 yrs	F	US W	PNW=78 POW=81	Non-CI	EAH-lab	CS-CC(P)	Significant group*age interaction (girls of OW mothers had greater increases in EAH from 5-9 yrs than girls of NW mothers; girls of OW mothers had greater increases in BMI from 5-9 years than girls of NW mothers).
Frances et al., 2007	168-197	5,7,9,11,13 yrs	F	US W	OW (at each age point) = 6%,11%,14%,14%,11%	Non-CI	EAH-lab	CS-CC(P)	Significant group difference in EAH (girls with two OW parents had higher EAH scores at 7,9,11 & 13 yrs than girls with one OW parent or none). Significant group*age interaction (girls with two OW parents had much greater increases on EAH from 7-13 years than girls with one OW parent or none; girls with two OW parents had greater increases in BMI from 5-9 years than girls of with one OW parent or none).

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight status[c]	CI/ Non-CI[d]			
Hill et al., 2008 - Study 1	348	7-9 yrs	M=170 F=178	UK W=47% non-W=53%	UW=56 NW=229 OW=44 OB=19	Non-CI	EAH-school	CS-CC(A) CS-A	Significant linear trend across weight groups for boys. Significant quadratic trend across weight groups (girls). Significant association (BMI & EAH) for boys but not for girls.
Hill et al., 2008, - Study 2	316	9-12 yrs	M=124 F=192	UK W=92% non-W=8%	UW=20 NW=227 OW=69 OB=21	Non-CI	EAH-home	CS-CC(A) CS-A	Significant linear trend across weight groups for boys. Significant quadratic trend across weight groups (girls). Significant association (BMI & EAH) for boys but not for girls.
Moens & Braet, 2007	52	7-13 yrs	M=16 F=36	Belgian	NW=26 OW=26	CI	EAH-home	CS-CC	Significant group difference for boys but not for girls.
Rodin & Slochower, 1976	92	9-15 yrs	F	US	NW=92	Non-CI	EAH-camp	L-A	Significant group difference (High EAH or low EAH & weight gain). Significant longitudinal association (EAH & weight gain).
Shomaker et al., 2010	78	13-17 yrs	M=44 F=34	US W=47% non-W=53%	NW=50 OW=28	Non-CI	EAH-lab	CS-CC CS-A	Significant group difference (OW children had higher scores). Significant association (higher EAH & higher BMI)
Shunk & Birch, 2004	153	5,7,9 yrs	F	US W=100%	NW=121 OW=32	Non-CI	EAH-lab	L-CC CS-CC	Significant group*age interaction (increases in EAH greater over time from 5-9 yrs for OW group). Significant weight group differences (age 7 & age 9).

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight status[c]	CI/ Non-CI[d]			
Epstein et al., 2008	65	8-12 yrs	M=32 F=33	US W=29% non-W=71%	NW=35 OW=30	Non-CI	Habituation & sensitisation - behavioural	CS-CC	Significant group*schedule interaction (OW habituated slower). Significant group*sensitisation*scedule interaction (sensitisation moderated rate of habituation for OW). Significant group difference for energy intake.
Epstein et al., 2009	84	8-12 yrs	M=42 F=42	US W=24% non-W=76%	NW=79% OW=21%	Non-CI	Habituation - behavioural	CS-A CS-CC	Significant BMI*schedule interaction (higher BMI = slower habituation rate). Significant group*food-type*schedule interaction (increased energy intake in OW group with variety foods compared to OW with same foods or NW with variety or same foods).
Hill et al., 2009a	316	7-9 yrs	M=158 F=158	UK W=46% non-W=54%	UW= 16%;12% NW= 65%;66% OW=14%;17% OB = 5%;5%	Non-CI	Food reinforcement - questionnaire	CS-CC(A) L-A	No cross-sectional association at baseline or follow-up (BMI & reinforcing value of food). Significant longitudinal association (baseline reinforcing value of food & increases in BMI, BMI SDS & fat mass).
Temple et al., 2008 - study 1	45	8-12 yrs	M=25 F=20	US	NW=25 OW=20	Non-CI	Food reinforcement - behavioural	CS-A CS-CC	Significant BMI*reinforcement schedule interaction (heavier children made significantly more responses for food as schedules progressed). Significant group difference for energy intake (OW consumed more).

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight status[c]	CI/ Non-CI[d]			
Temple et al., 2008 - study 2	45	8-12 yrs	M=22 F=23	US	NW=22 OW=23	Non-CI	Food reinforcement - behavioural	CS-CC	Significant group*reinforcer*reinforcement schedule interaction (OW found food more reinforcing than non-food; NW found non-food more reinforcing than food). Significant group difference for energy intake (OW consumed more).
								CS-A	Same interaction observed using BMI instead of weight group.
Temple et al., 2007	34	8-10 yrs	M=16 F=18	US W=59% non-W=41%	NW=17 OW=17	Non-CI	Habituation - behavioural	CS-CC	Significant group* schedule interaction (OW children made more responses/ 2 mins than NW children, as schedule progressed). Significant group difference for energy intake (OW consumed more).
Braet & van Strien, 1997	292	9-12 yrs	M=110 F=181	Dutch	NW=147 OB=145	CI	DEBQ-P – 'external eating'	CS-CC	Significant group difference (OB children had higher scores).
Braet et al., 2008	2474	7-18 yrs	M=1094 F=1380	Beligian	NW=1200 OB=1274	CI	DEBQ-C – 'external eating'	CS-CC	Significant group difference (OW children had lower scores).
Caccialanza et al., 2004	312	11-14 yrs	M=158 F=154	Italian	NW=240 OW=59 OB=13	Non-CI	DEBQ-P – 'external eating'	CS-CC	No significant difference across weight groups.
Carnell & Wardle, 2008a - study 1	10364	8-11 yrs	M=5024 F=5340	UK W=93% non-W=7%	NW=86% OW=11% OB=3%	Non-CI	CEBQ-EF	CS-A	Significant association (higher BMI & higher EF scores; higher waist circumference & higher EF scores).
								CS-CC(A)	Significant weight group difference & significant linear effect across weight groups for BMI & waist circumference.

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight status[c]	CI/ Non-CI[d]			
Carnell & Wardle, 2008a - study 2	572	3-5 yrs	M=304 F=268	UK W=73% non-W=27%	NW=424 OW=108 OB=40	Non-CI	CEBQ-EF	CS-A CS-CC(A)	Significant association (higher BMI & higher EF scores). Significant weight group difference & significant linear effect across weight groups for BMI & EF scores, and BMI & FR scores.
Cunha et al., 2010	321	9-12 yrs	M=156 F=165	Portuguese	UW=5 NW=204 OW=61 OB=51	Non-CI	CEBQ-FR CEBQ-EF	CS-A CS-CC(A)	Significant association (higher BMI & higher FR scores; higher BMI & higher EF scores). Appeared to be linear effect across weight groups for FR & EF (although linear tests not reported).
Gregory et al., 2010	156	2-4 yrs	M=77 F=79	Australian	NW=85% OW=15%	Non-CI	CEBQ-FR	CS-A L-A	Significant cross-sectional association at time 1/ age 3 (higher BMI & higher FR scoresl). Not significant cross-sectional association at time 2/ age 4 (BMI & FR scores). No significant longitudinal association (T1 FR score did not significant predict change in BMI from T1 to T2).
Halford et al., 2004	42	9-11 yrs	M=18 F=24	UK	NW=67% OW=21% OB=12%	Non-CI	DEBQ – 'external eating'	CS-CC	No significant group difference.
Hill et al., 1994	379	9yrs	M=166 F=213	UK W=80% Non-W=20%	UW=126 NW=127 OW=64 OB=62	Non-CI	DEBQ – 'external eating'	CS-CC(A)	No significant group difference.

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight status[c]	CI/ Non-CI[d]			
Jahnke & Warschburger, 2008	142	3-6 yrs	M=91 F=51	German	UW=4.2% NW=76.8% OW=12% OB=7%	CI (psyco-pathology)	DEBQ – 'external eating' CEBQ-FR	CS-CC CS-CC(P)	Significant group difference for 'external eating' (UW/NW lower than OW/OB). Significant group difference for FR (UW/NW lower than OW/OB). No significant group difference by maternal weight status.
Joyce & Zimmer-Gembeck, 2009	211	4-8 yrs	M=110 F=101	Australian W=94% non-W=6%	NW=87.2% AROW=8.1% OW=4.7%	Non-CI	CEBQ-FR & CEBQ-EOE combined	CS-A	Significant association (higher BMI & higher combined scale scores).
Dubois et al., 2007a	1498	2.5,3.5,4.5 yrs	M=756 F=742	Canadian	-[NR]	Non-CI	Parental-report 'overeating' item	CS-CC	Significant association (overeaters at 2.5 or 3.5 yrs more likely to be OW at 4.5 yrs).
Engel & Zeitlin, 1996	80	12-19 mths		Nicaraguan	UW=22 NW=58	Non-CI	Child Demand Scale	CS-A	Significant association (higher weight & higher demand for botte-feeds).
Li et al., 2008	1896	≤ 6 mths	M=891 F=1005	US W=84% H=9% B=7%	NW (z score ≤1)=1650 OW (z-score>1)=246	Non-CI	Bottle-emptying item	L-CC	Significant association (greater frequency of bottle-emptying during 1st 6 mths in infants who were OW during 2nd 6 mths).
Parkinson et al., 2010	344	6 wks 1 yr 5-6 yrs 6-8 yrs	M=289 F=294	UK	Non-GF=90% GF=6% S-GF=4%	Non-cl	CEBQ-FR CEBQ-EF GA	CS-CC(A) L-A	Significant linear effect across weight groups (FR & EF increased linearly in each BMI tertile). Significant longitudinal association (higher GA at 5-6 yrs & higher BMI at 7-8 yrs. No significant longitudinal association (GA at 6 wks & BMI at 7-8 yrs; GA at 12 mths & BMI at 7-8 yrs). No significant longitudinal association (FR at 5-6 yrs & BMI at 7-8 yrs; EF at 5-6 yrs & BMI at 7-8 yrs).

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight status[c]	CI/ Non-CI[d]			
Powers et al., 2006	296	2-5 yrs	M=154 F=142	US B=100%	UW=19 NW=189 OW=44 OB=38	Non-CI	CEBQ-FR	CS-CC(A)	No significant group difference (weight group & FR).
Sleddens et al., 2008	135	6-7 yrs	M=68 F=67	Dutch	UW=20 NW=83 OW=12	Non-CI	CEBQ-FR CEBQ-EF	CS-A CS-CC(A)	Significant association (higher BMI & higher FR; higher BMI & higher EF). No significant difference between weight groups.
Spence et al., 2010	1730	4-5 yrs	M=884 F=846	Canadian	UW=69 NW=1254 AROW=256 OW=151	Non-CI	CEBQ-FR CEBQ-EF	CS-CC(A)	Significant weight group difference & significant linear effect across weight groups for BMI & EF scores, and BMI & FR scores (higher scores in higher weight groups, lower scores in lower weight groups).
Van Strien & Oosterveld, 2008	769	7-12 yrs	M=382 F=387	Dutch	NW=626 OW=143	Non-CI	DEBQ-C – 'external eating'	CS-CC	No significant group difference.
Viana et al., 2008	256	3-13 yrs	M=117 F=123	Portuguese	UW=10 NW=116 OW=46 OB=84	CI	CEBQ-FR CEBQ-EF	CS-A CS-CC(A)	Significant association (higher BMI & higher FR scores; higher BMI & higher EF scores). Significant linear associations across weight groups for FR scores & EF scores.
Wardle et al., 1992	846	11-18 yrs	M=407 F=439	UK W=63% non-W=37%	-[NR]	Non-CI	DEBQ – 'external eating'	CS-A	Significant association (Higher BMI & lower 'external eating' scores).
Webber et al., 2009	406	7-12 yrs	M=188 F=218	UK W=70% Non-W=30%	UW=42 NW=282 OW=62 OB=20	Non-CI	CEBQ-FR CEBQ-EF	CS-A CS-CC(A)	Significant association (higher BMI & higher EF scores; higher BMI & higher FR scores). Significant linear effect across weight groups for BMI & FR, & BMI & EF.

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight status[c]	CI/ Non-CI[d]			
Wright et al., 2006	749	6 wks, 4,8,12 mths	-[NR]	UK W	Non-GF=90% GF=6% S-GF=4%	Non-CI	GA	CS-A	Significant association ('appetite' at 6 wks & weight gain 0-6 wks; 'appetite' at 6 wks & weight faltering 0-6 wks; 'appetite' at 12 mths & weight gain 0-12 mths).
								L-A	Significant longitudinal association ('appetite' at 6 wks & weight gain 0-12 mths, & sustained weight faltering at 12 mths).

[a] Only the abstract was available so much of the information was not accessible.
[b] W, 'white'; non-W, 'non-White'; H, 'Hispanic'; B, 'Black'.
[c] -[NR], not reported; UW, 'underweight', NW, 'normal weight'; OW, 'overweight'; OB, 'obese'; PNW, 'parental normal weight'; POW, 'parental overweight'; AROW, 'at risk for overweight'; non-GF, no growth faltering during the first year of life; GF, growth faltering at some point during the first year of life; SGF, sustained growth faltering for the first year of life.
[d] CI, clinical sample of overweight children; non-CI, non-clinical sample of overweight children; CI (psychopathology), mother or child had diagnosed psychopathology.
[e] SAE, 'sensory activation of eating paradigm' (SAE-lab, experiment took place in a laboratory setting); EAH, 'eating in the absence of hunger' (EAH-lab, experiment took place in a laboratory setting; EAH-school, experiment took place in school; EAH-home, experiment took place in children's homes; EAH-camp, experiment took place at summer holiday camp); DEBQ, Dutch Eating Behaviour Questionnaire; DEBQ-P, Dutch Eating Behaviour Questionnaire, parent-report version; DEBQ-C, Dutch Eating Behaviour Questionnaire, child-report version; CEBQ, Child Eating Behaviour Questionnaire (CEBQ-EF, 'enjoyment of food' scale; CEBQ-FR, 'food responsiveness' scale; CEBQ-EOE, 'emotional overeating' scale); GA, 'general appetite' item ('At present, how is your baby/child's appetite?').
[f] CS-CC, cross-sectional case-control design using child overweight/ normal weight categorisation; CS-CC(A), cross-sectional case-control design using more than two weight categories in order to explore relationship with weight spectrum; CS-CC(P), cross-sectional case-control design using parental overweight/ normal weight categoristation; CS-A, cross-sectional design using continual association; L-A, longitudinal design using continual association; L-CC, longitudinal case control design with two weight groups (NW and OW).

Appendix 1.2. Measures of internal satiety sensitivity and weight

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight Status[c]	CI/ Non-CI[d]			
Agras et al., 1987	99	2 & 4 wks 1 & 2 yrs	M=51 F=48	US W=86% H=6% B=2%	-[NR]	Non-CI	SoE – lab (Kron)	L-A	Significant longitudinal association (shorter interburst sucking interval at 2-4 wks & higher BMI at 1 yr; higher sucking pressure at 2-4 wks & higher BMI at 2 yrs).
Agras et al., 1990, 2004	54	2 & 4 wks 3, 6, 9 yrs	M=51 F=48	US W=86% H=6% B=2%	-[NR]	Non-CI	SoE – lab (Kron)	L-A	No significant longitudinal association (sucking pressure at 2-4 wks & BMI at 3, 6 & 9 yrs).
Barkeling et al., 1992[a]	43	11 yrs	-	Swedish	NW=23 OB=20	Non-CI	SoE – lab (g/sec) Deceleration (lab)	CS-CC	Significant group difference for eating rate (OB ate faster). Significant group difference for deceleration (more deceleration in NW group).
Berkowitz et al., 2010	61	4 & 6 yrs	M=31 F=30	US W=100%	PNW=29 POW=32	Non-CI	SoE – lab (mouthfuls/min , kcals/min, meal duration)	L-A	Significant longitudinal association (increased mouthfuls/min at 4yrs predicted +ve change in BMI, skinfold thickness, fat & %fat from 4-6 yrs).
								L-CC	Significant longitudinal association (increased kcal/min, increased mouthfuls/min & shorter meal duration at 4yrs predicted increased OW / OB at 6 yrs).
								CS-CC(P)	Significant difference in mouthfuls/min & meal duration between OW/OB & NW groups within POW children at 6 yrs.
Drabman et al., 1979	60	1.5-6 yrs	-[NR]	US W=100%	NW=30 OW=30	Non-CI	SoE – school cafeteria (bites/30 secs)	CS-CC	Significant group difference (OW took more bites/ 30 secs).

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight Status[c]	CI/ Non-CI[d]			
Epstein et al., 1976	6	7 yrs	M=3 F=3	US	NW=3 OB=3	Non-CI	SoE – school cafeteria (bites/10 secs)	Intervention	Significant decrease in bite rate over 6 months following intervention.
Israel et al., 1985	60	7-12 yrs	M=24 F=36	US	NW=40 OB=20	Non-CI	SoE – school cafeteria (bites/30 secs)	CS-CC	No significant association (bite rate & % overweight). No significant group difference (bite rate).
Keane et al., 1981	20	10-11 yrs	M=10 F=10	US	NW=10 OB=10	Non-CI	SoE – school classroom	CS-CC	Significant group difference (OB took more bites/30 secs).
Laessle at al., 2001	80	8-12 yrs	M=44 F=36	German	NW=42 OB=38	Non-CI	SoE – lab (g/sec) Deceleration (lab)	CS-CC	Significant group difference (OB ate faster when mother present). Significant group difference (OB accelerated towards end of meal when mother present).
Lindgren et al., 2000	40	5-18 yrs	M=19 F=21	Swedish	NW=20 OB=20	CI	SoE – lab (g/min) Deceleration (lab)	CS-CC	No significant group difference for eating rate. Significant group difference for deceleration (more deceleration in NW).
Llewellyn et al., 2008	254	8-11 yrs	M=102 F=152	UK W=100%	NW=186 OW/OB=68	Non-CI	SoE – home (bites/min)	CS-A CS-CC(A)	Significant association (higher eating rate & higher BMI). Significant group difference and significant linear trend across groups.
Stunkard et al., 2004	78	3mths 2yrs	M=39 F=39	US	PNW=38 POW=40	Non-CI	SoE – lab (similar to Kron)	CS-CC(P) CS-A(P) L-A	Significant group difference (POW took more total sucks than PNW, but feed duration the same). Significant association (higher number of sucks & higher maternal BMI) Significant longitudinal association (higher number of sucks at 3 mths & weight gain from 3 mths to 2 yrs).

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight Status[c]	CI/ Non-CI[d]			
Waxman & Stunkard, 1980	8	4-12 yrs	M	US W=25% H=25% B=50%	NW=4 OB=4	Non-CI	SoE – school	CS-CC	Significant group difference (OB ate faster than NW brothers).
Carnell et al., unpublished data	77	3-5 yrs	-	UK W	-	Non-CI	Preload 20 mins – low energy, high energy (liquids)	CS-A	Significant association (fatter children had poorer compensation abilities).
Cecil et al., 2005	74	6-9 yrs	M=37 F=37	UK – Scotland W	NW=57 OW=11 OB=6	Non-CI	Preload 90 mins – no energy, low energy, high energy (solids)	CS-A	No significant association (compensation ability & BMI).
Faith et al., 2004	32	3-7 yrs	M=16 F=21	US W=25% B=40% H=25% Other=10%	NW=37 OW=7	Non-CI	Preload 25 mins – low energy, high energy (liquids)	CS-A	No significant association (compensation ability & BMI).
Jansen et al., 2003	31	8-12 yrs	M=17 F=14	Dutch	NW=15 OW=16	Non-cl	Preload 10 mins – high energy (solids)	CS-CC	Significant group difference (NW children ate significant less after preload but OW children did not).
Johnson & Birch, 1994	77	3-5 yrs	M=31 F=46	US W=82% Non-W=18%	-[NR]	Non-CI	Preload 20 mins – low energy, high energy (liquids)	CS-A	Significant association for girls (fatter girls with greater skinfold thickness had poorer compensation abilities). No significant association for boys.
Johnson & Taylor-Holloway, 2006	262	5-11 yrs	M=126 F=136	US W=46% H=54%	NW=224 OW=38	Non-CI	Preload 30 mins – low energy, high energy (liquids)	CS-A	No significant association.

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight Status[c]	CI/ Non-CI[d]			
Kasese-Hara et al., 2002	53	12-24 mths	M=29 F=24	UK	UW=27 NW=26	CI (UW) & non-CI (NW)	Preload 25 mins – low energy, high energy (liquids) - home	CS-CC	Significant difference (intake at meal lower in UW than NW following low energy & high energy drinks; intake of preload lower in UW than NW; BUT UW consumed significant more after high energy preload while NW compensated).
Carnell & Wardle – study 1, 2008a	10364	8-11 yrs	M=5024 F=5340	UK W=93% non-W=7%	NW=86% OW=11% OB=3%	Non-cl	CEBQ-SR & CEBQ SE combined	CS-A CS-CC(A)	Significant association (higher BMI & lower SE/SR scores; higher waist circumference & lower SE/SR scores). Significant weight group difference & significant linear effect across weight groups for BMI & SE/SR scores, and waist circumference & SE/SR scores.
Carnell & Wardle – study 2, 2008a	572	3-5 yrs	M=304 F=268	UK W=73% non-W=27%	NW=424 OW=108 OB=40	Non-cl	CEBQ-SR & CEBQ SE combined	CS-A CS-CC(A)	Significant association (higher BMI & lower SE/SR scores). Significant weight group difference & significant linear effect across weight groups for BMI & SE/SR scores.
Cunha et al., 2010	321	9-12 yrs	M=156 F=165	Portuguese	UW=5 NW=204 OW=61 OB=51	Non-cl	CEBQ-SE CEBQ-SR	CS-A CS-CC(A)	Significant association (higher BMI & lower SR scores; higher BMI & lower SE scores). Appeared to be linear effect across weight groups (although linear tests not reported).
He at al., 2000	1322	0-.1-7 yrs	M=748 F=574	Chinese	NW=661 OB=661	Non-CI	Eating Speed (1 item)	CS-CC	Significant association (eating speed predicted NW/OB).
Jahnke & Warschburger, 2008	142	3-6 yrs	M=91 F=51	German	UW=4.2% NW=76.8% OW=12% OB=7%	CI (psyco-pathology)	Eating Speed (4-items)	CS-CC CS-CC(P)	Significant group difference for eating speed (UW/NW lower eating speed than OW/OB). No significant group difference by maternal weight status.

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight Status[c]	CI/ Non-CI[d]			
Parkinson et al., 2010	344	6 wks 1 yr 5-6 yrs 6-8 yrs	M=289 F=294	UK	Non-GF=90% GF=6% S-GF=4%	Non-cl	CEBQ-SR CEBQ-SE	CS-CC(A) L-A	Significant linear effect across weight groups (SR & SE increased in each BMI tertile). Significant longitudinal association (higher SR at 5-6 yrs & lower BMI at 7-8 yrs). No significant longitudinal association with SE & BMI.
Sleddens et al., 2008	135	6-7 yrs	M=68 F=67	Dutch	UW=20 NW=83 OW=12	Non-cl	CEBQ-SE CEBQ-SR	CS-A CS-CC(A)	Significant association for SR & SE (higher BMI & lower SR; higher BMI & lower SE). Significant weight group differences for BMI & SR scores, & BMI & SE scores.
Spence et al., 2010	1730	4-5 yrs	M=884 F=846	Canadian	UW=69 NW=1254 AROW=256 OW=151	Non-CI	CEBQ-SR CEBQ-SE	CS-CC(A)	Significant weight group difference & significant linear effect across weight groups for BMI & SR scores, and BMI & SE scores (higher scores in lower weight groups, lower scores in higher weight groups).
Sugimori et al., 2004	7693	3yrs 6yrs	M=3923 F=3770	Japanese	(3yrs/6yrs) NW/NW=6404 OB/NW=482 NW/OB=474 OB/OB=333	Non-CI	Eating Speed (1 item)	L-CC	Significant association (eating speed predicted change from NW to OB from 3yrs to 6 yrs, & maintaining OB from 3 yrs to 6 yrs).
Viana et al., 2008	256	3-13 yrs	M=117 F=123	Portuguese	UW=10 NW=116 OW=46 OB=84	CI	CEBQ-SE CEBQ-SR	CS-A CS-CC(A)	Significant association (higher BMI & lower SR scores; higher BMI & lower SE scores). Significant linear associations across weight groups for SR & SE scores.

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight Status[c]	CI/ Non-CI[d]			
Webber et al., 2009	406	7-12 yrs	M=188 F=218	UK W=70% Non-White=30%	UW=42 NW=282 OW=62 OB=20	Non-cl	CEBQ-SE CEBQ-SR	CS-A CS-CC(A)	Significant association (higher BMI & lower SR scores; higher BMI & lower SE scores). Significant linear effect across weight groups for BMI & SR, & BMI & SE.

[a] Only the abstract was available so much of the information was not accessible.
[b] W, 'white'; non-W, 'non-White'; H, 'Hispanic'; B, 'Black'.
[c] -[NR], not reported; UW, 'underweight', NW, 'normal weight'; OW, 'overweight'; OB, 'obese'; PNW, 'parental normal weight'; POW, 'parental overweight'; AROW, 'at risk for overweight'; non-GF, no growth faltering during the first year of life; GF, growth faltering at some point during the first year of life; SGF, sustained growth faltering for the first year of life.
[d] CI, clinical sample of overweight children; non-CI, non-clinical sample of overweight children; CI (psychopathology), mother or child had diagnosed psychopathology.
[e] SoE-lab (Kron), speed of feeding measured in a laboratory used Kron's sucking apparatus; SoE-lab, speed of eating measured in a laboratory using amount consumed per period of time; SoE-school cafeteria/ classroom, speed of eating measured in a school setting using bites taken per period of time; SoE-home, speed of eating measured at the child's home using bites taken per period of time; CEBQ, Child Eating Behaviour Questionnaire (CEBQ-SR, 'satiety responsiveness' scale; CEBQ-SE, 'slowness in eating' scale).
[f] CS-CC, cross-sectional case-control design using child overweight/ normal weight categorisation; CS-CC(A), cross-sectional case-control design using more than two weight categories in order to explore relationship with weight spectrum; CS-CC(P), cross-sectional case-control design using parental overweight/ normal weight categoristation; CS-A, cross-sectional design using continual association; CS-A(P), cross-sectional design using continual association between child/infant feeding behaviour and parental adiposity; L-A, longitudinal design using continual association; L-CC, longitudinal case control design with two weight groups (NW and OW).

Appendix 1.3. Measures of food preferences and 'food fussiness' and weight

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight Status[c]	CI/ Non-CI[d]			
Fieldstone et al., 1997	26	12-22 yrs	M=14 F=12	US	NW=14 OW=12	Non-CI	Taste test & ranking – high carb; high fat; high protein foods	CS-CC	No significant weight group difference (preference for high fat foods).
Fisher & Birch, 1995	18	3-5 yrs	M=8 F=10	US	NW=14 OW=4	Non-CI	Taste test & ranking – high fat & low fat foods	CS-A(P) CS-A	Significant association (higher parental BMI & higher preference for high fat foods). Significant association (higher triceps skinfold & higher preference for high fat foods).
Halford et al., 2008	37	11-13 yrs	M=20 F=24	UK	NW=24 OW=10 OB=3	Non-CI	Food preferences questionnaires – high carb; high fat; high protein; high energy density foods Forced food choice	CS-CC	Significant interaction of weight group*food branding (OW preferred more branded than unbranded high fat foods; NW preferred more unbranded than branded high carbohydrate foods). No significant weight group effect on forced food choice.
Hill et al., 2009b	366	7-9 yrs	M=185 F=181	UK W=46% Non-W=54%	UW=56 NW=243 OW=49 OB=18	Non-CI	Food preferences questionnaire – fruits & vegetables; fatty & sugary foods	CS-A CS-CC(A)	No significant association (BMI & liking for any type of food). No significant weight group effect.
Lakkakula et al., 2008	341	10-11 yrs	M=147 F=194	US B	UW=10 NW=205 OW=58 OB=68	Non-CI	Food preferences questionnaire – fruits & vegetables	CS-A CS-CC	Significant association (lower BMI & higher preference score for fruits & vegetables. Significant weight group difference (lower preference for fruits & vegetables in OW).

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight Status[c]	CI/ Non-CI[d]			
Ricketts, 1997	88	9-12 yrs	M=51 F=37	US	-[NR]	Non-CI	Taste test & ranking – regular snack foods & low-fat equivalents	CS-A	Significant association (higher BMI & higher fat preference; higher tricep skinfold thickness & higher fat preference). No significant association (subscapular skinfold thickness & fat preference).
Wardle et al., 2001a	428	4-5 yrs	M=205 F=223	UK	PNW=228 POW=200	Non-CI	Food preferences parent-report questionnaire – meats, sweet deserts, fruits, vegetables. Taste test & ranking – high fat & low fat foods.	CS-CC(P)	Significant weight group difference (POW had higher fat preferences & lower vegetables preferences). No significant group differences for protein foods, sweet desserts or fruits.
Xiong et al., 2008[a]	5755	6-19 yrs	-	Chinese	NW=2136 OW=1947	Non-CI	Food preferences questionnaire – range of common foods	CS-CC	Significant weight group difference (higher preference for vegetables associated with lower odds for OW).
Carruth et al., 1998	118	2,3,4,5,6,7 yrs	-[NR]	US W	-[NR]	Non-CI	'Picky eating' questionnaire	CS-CC	No significant weight difference between 'picky' & 'non-picky' eaters at any age.
Carruth et al., 2004	3022	4-24 mths	M=1541 F=1481	US W=77% B=7% H=10% Other=6%	-[NR]	Non-CI	'Picky eater' item	CS-CC(A)	Significant association (children in top 3 quartiles of weight less likely to be picky eaters).
Cunha et al., 2010	321	9-12 yrs	M=156 F=165	Portuguese	UW=5 NW=204 OW=61 OB=51	Non-CI	CEBQ-FF	CS-A CS-CC(A)	Significant association (lower BMI & higher FF scores). There did not appear to be a linear effect across weight groups (although linear tests not reported).

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight Status[c]	CI/ Non-CI[d]			
Dubois et al., 2007a	1498	2.5,3.5,4.5 yrs	M=756 F=742	Canadian	UW=15.2% NW=64.4% AROW=11.3% OW=9.0%	Non-CI	Parental-report 'picky eating' item	CS-CC	Significant association ('picky eaters' at all ages were more likely to be UW at 4.5 yrs).
Ekstein et al., 2010	170	14-91 mths	M=120 F=50	Israeli	UW=9% NW=91%	CI & Non-CI	'Picky eaters' in treatment	CS-CC	Significant association ('picky eaters' more likely to be UW).
Galloway et al., 2005	173	9 yrs	F	US W	-[NR]	Non-CI	Child Feeding Questionnaire – 'picky eating' scale	CS-CC	Significant difference ('picky eaters' had lower BMIs & body fat than 'non-picky' eaters). Significant association (fewer picky eaters were OW or OB).
Gregory et al., 2010	156	2-4 yrs	M=77 F=79	Australian	NW=85% OW=15%	Non-CI	CEBQ-FF	CS-A L-A	No significant cross-sectional association at time 1/ age 3. Not significant cross-sectional association at time 2/ age 4. No significant longitudinal association (T1 FF score did not significant predict change in BMI from T1 to T2).
Parkinson et al., 2010	344	6 wks 1 yr 5-6 yrs 6-8 yrs	M=289 F=294	UK	Non-GF=90% GF=6% S-GF=4%	Non-cl	CEBQ-FF	L-A	No significant longitudinal association (FF at 5-6 yrs & BMI at 7-8 yrs).
Rydell et al., 1995[a]	240	Primary school age	-	Swedish	-	Non-CI	'choosiness' questionnaire	CS-CC	No significant group difference (BMI of 'choosy' or 'non-choosy').
Sleddens et al., 2008	135	6-7 yrs	M=68 F=67	Dutch	UW=20 NW=83 OW=12	Non-CI	CEBQ-FF	CS-A CS-CC(A)	No significant association for FF & BMI. No significant weight group difference for FF.

Author & year[a]	Sample Characteristics						Measure[e]	Design[f]	Findings
	n	Age	Sex[c]	Nationality / Ethnicity[b]	Weight Status[c]	Cl/ Non-Cl[d]			
Spence et al., 2010	1730	4-5 yrs	M=884 F=846	Canadian	UW=69 NW=1254 AROW=256 OW=151	Non-Cl	CEBQ-FF	CS-CC(A)	Significant weight group difference & significant linear effect across weight groups for BMI & FF scores (lower scores in higher weight groups, higher scores in lower weight groups).
Viana et al., 2008	256	3-13 yrs	M=117 F=123	Portuguese	UW=10 NW=116 OW=46 OB=84	Cl	CEBQ-FF	CS-A CS-CC(A)	Significant association (higher BMI & lower FF scores). No significant linear associations across weight groups for FF.
Webber et al., 2009	406	7-12 yrs	M=188 F=218	UK W=70% Non-White=30%	UW=42 NW=282 OW=62 OB=20	Non-Cl	CEBQ-FF	CS-A CS-CC(A)	Significant association for girls but not for boys (higher BMI & lower FF scores). Significant linear effect across weight groups for girls FF.
Wright et al., 2007	455	29-33 mths	-[NR]	UK W	Non-GF=90% GF=6% S-GF=4%	Non-Cl	'faddy eating' scale	CS-CC L-A	No significant group difference (weight of 'faddy' and 'non-faddy'). No significant association (growth of 'faddy' and 'non-faddy' from 0-2 yrs or 1-2 yrs).

[a] Only the abstract was available so much of the information was not accessible.
[b] W, 'white'; non-W, 'non-White'; H, 'Hispanic'; B, 'Black'.
[c] -[NR], not reported; UW, 'underweight', NW, 'normal weight'; OW, 'overweight'; OB, 'obese'; PNW, 'parental normal weight'; POW, 'parental overweight'; AROW, 'at risk for overweight'; non-GF, no growth faltering during the first year of life; GF, growth faltering at some point during the first year of life; SGF, sustained growth faltering for the first year of life.
[d] Cl, clinical sample of overweight children; non-Cl, non-clinical sample of overweight children.
[e] CEBQ-FF, Child Eating Behaviour Questionnaire 'food fussiness' scale.[f]CS-CC, cross-sectional case-control design using child overweight/ normal weight categorisation; CS-CC(A), cross-sectional case-control design using more than two weight categories in order to explore relationship with weight spectrum; CS-CC(P), cross-sectional case-control design using parental overweight/ normal weight categoristation; CS-A, cross-sectional design using continual association; CS-A(P), cross-sectional design using continual association between child/infant feeding behaviour and parental adiposity; L-A, longitudinal design using continual association.

Appendix 1.4. Measures of 'emotional eating' and weight

Author & year	Sample Characteristics						Measure[d]	Design[e]	Findings
	n	Age	Sex	Nationality / Ethnicity[a]	Weight status[b]	CI/ Non-CI[c]			
Braet & van Strien, 1997	292	9-12 yrs	M=110 F=181	Dutch	NW=147 OB=145	CI	DEBQ-P – 'emotional eating'	CS-CC	Significant group difference (OB children had higher scores).
Braet et al., 2008	2474	7-18 yrs	M=1094 F=1380	Beligian	NW=1200 OB=1274	CI	DEBQ-C – 'emotional eating'	CS-CC	No significant difference across weight groups.
Caccialanza et al., 2004	312	11-14 yrs	M=158 F=154	Italian	NW=240 OW=59 OB=13	Non-cl	DEBQ-P – 'emotional eating'	CS-CC	No significant difference across weight groups.
Cunha et al., 2010	321	9-12 yrs	M=156 F=165	Portuguese	UW=5 NW=204 OW=61 OB=51	Non-cl	CEBQ-EOE CEBQ-EUE	CS-A CS-CC(A)	Significant association (higher BMI & higher EOE scores; higher BMI & lower EUE scores). Appeared to be linear effect across weight groups for EOE (although linear test not reported). Less linearity appeared to be present for EUE.
Hill et al., 1994	379	9yrs	M=166 F=213	UK W=80% Non-W=20%	UW=126 NW=127 OW=64 OB=62	Non-CI	DEBQ – 'emotional eating'	CS-CC(A)	Significant group difference for girls (UW girls had highest scores and OW girls had lowest scores). No significant group difference for boys.
Jahnke & Warschburger, 2008	142	3-6 yrs	M=91 F=51	German	UW=4.2% NW=76.8% OW=12% OB=7%	CI (psyco-patho-logy)	DEBQ-P – 'emotional eating'	CS-CC CS-CC(P)	No significant group difference for 'emotional eating'. Significant group difference by maternal weight status (children of OW mothers had higher 'emotional eating' scores).
Jollie-Trottier et al., 2009	291	10-11 yrs	M=137 F=153	US American-Indian	UW=<1% NW=47% OW=20% OB=33%	Non-CI	DEBQ – 'emotional eating'	CS-A	No significant association.

Author & year	Sample Characteristics						Measure[d]	Design[e]	Findings
	n	Age	Sex	Nationality / Ethnicity[a]	Weight status[b]	CI/ Non-CI[c]			
Joyce & Zimmer-Gembeck, 2009	211	4-8 yrs	M=110 F=101	Australian W=94% non-W=6%	NW= OW=	Non-cl	CEBQ-FR & CEBQ-EOE combined	CS-A	Significant association (higher BMI & higher combined scale scores).
Parkinson et al., 2010	344	6 wks 1 yr 5-6 yrs 6-8 yrs	M=289 F=294	UK	Non-GF=90% GF=6% S-GF=4%	Non-cl	CEBQ-EOE CEBQ-EUE	L-A	Significant longitudinal association (higher EOE at 5-6 yrs & higher BMI at 7-8 yrs). No significant longitudinal association (EUE at 5-6 yrs & BMI at 7-8 yrs).
Sleddens et al., 2008	135	6-7 yrs	M=68 F=67	Dutch	UW=20 NW=83 OW=12	Non-cl	CEBQ-EOE CEBQ-EUE	CS-A CS-CC(A)	No significant association (BMI & EOE, or BMI & EUE). No significant difference between weight groups.
Spence et al., 2010	1730	4-5 yrs	M=884 F=846	Canadian	UW=69 NW=1254 AROW=256 OW=151	Non-CI	CEBQ-EOE CEBQ-EUE	CS-CC(A)	Significant weight group difference & significant linear effect across weight groups for BMI & EOE scores (higher scores in higher weight groups). No significant difference for EUE.
Striegel-Moore et al., 1999	2379	9-10 yrs	F	US W=49% B=51%	-[NR]	Non-CI	'Emotion-induced eating' scale (7 items)	CS-A	Significant association (higher BMI and lower 'emotion-induced eating').
Van Strien & Oosterveld, 2008	769	7-12 yrs	M=382 F=387	Dutch	NW=626 OW=143	Non-CI	DEBQ-C – 'emotional eating'	CS-CC	No significant group difference.
Viana et al., 2008	256	3-13 yrs	M=117 F=123	Portuguese	UW=10 NW=116 OW=46 OB=84	CI	CEBQ-EOE CEBQ-EUE	CS-A CS-CC(A)	Significant association (higher BMI & higher EOE scores; higher BMI & lower EUE scores). Significant linear associations across weight groups for EOE scores but not for EUE scores.
Wardle et al., 1992	846	11-18 yrs	M=407 F=439	UK W=63% non-W=37%	-[NR]	Non-CI	DEBQ – 'emotional eating'	CS-A	No significant association (BMI & 'emotional eating' scores).

Author & year	Sample Characteristics						Measure[d]	Design[e]	Findings
	n	Age	Sex	Nationality / Ethnicity[a]	Weight status[b]	Cl/ Non-Cl[c]			
Webber et al., 2009	406	7-12 yrs	M=188 F=218	UK W=70% Non-W=30%	UW=42 NW=282 OW=62 OB=20	Non-cl	CEBQ-EOE CEBQ-EUE	CS-A CS-CC(A)	Significant association for EOE & BMI (higher BMI & higher EOE). No significant association for EUE & BMI. Significant linear effect across weight groups for BMI & EOE scores but not for EUE scores.

[a] W, 'white'; non-W, 'non-White'; B, 'Black'.

[b] -[NR], not reported; UW, 'underweight', NW, 'normal weight'; OW, 'overweight'; OB, 'obese'; AROW, 'at risk for overweight'; non-GF, no growth faltering during the first year of life; GF, growth faltering at some point during the first year of life; SGF, sustained growth faltering for the first year of life.

[c] Cl, clinical sample of overweight children; non-Cl, non-clinical sample of overweight children; Cl (psychopathology), mother or child had diagnosed psychopathology.

[d] DEBQ, Dutch Eating Behaviour Questionnaire; DEBQ-P, Dutch Eating Behaviour Questionnaire parent-report version; DEBQ-C, Dutch Eating Behaviour Questionnaire child-report version; CEBQ, Child Eating Behaviour Questionnaire (CEBQ-EOE, 'emotional overeating' scale; CEBQ-EUE, 'emotional under-eating' scale; CEBQ-FR, 'food responsiveness' scale).

[e] CS-CC, cross-sectional case-control design using child overweight/ normal weight categorisation; CS-CC(A), cross-sectional case-control design using more than two weight categories in order to explore relationship with weight spectrum; CS-CC(P), cross-sectional case-control design using parental overweight/ normal weight categoristation; CS-A, cross-sectional design using continual association; L-A, longitudinal design using continual association.

Appendix 1.5. Eating behaviour differences in children or infants who 'fail to thrive'

Author & year[a]	Sample Characteristics					Measure	Design[e]	Findings
	n	Age	Sex[b]	Nationality / Ethnicity[c]	FTT status[d]			
Drewett et al., 2003	56	12-24 mths	M=28 F=28	UK	FTT=28 Non-FTT=28	Behavioural observation	CS-CC	Significant difference (FTT less energy intake, shorter meal duration, more food refusal, more rejection of food).
Garcia et al., 1990[a]	45	33-60 mths	-	Mexican	FTT	Behavioural observation (1 day)	Qualitative	Children had free access to 2029 kcals but only consumed 1528 demonstrating child's role in determining intake (or lack of intake).
Parkinson et al., 2004	87	13-21 mths	M=45 F=42	UK W	FTT=30 Non-FTT=57	Behavioural observation (2 meals)	CS-CC	Significant difference (FTT less energy intake). No significant group differences (giving food, accepting food, feeding self, refusing food, rejecting food, meal duration).
Wilensky et al., 1996	1407	25 mths	-[NR]	Israeli	FTT=55 Non-FTT=1352	Structured interview to establish feeding problems	CS-CC	Significant difference (FTT showed less hunger, closed mouth & turned head more, showed less pleasure at mealtimes, more nervous at mealtimes, ate less variety of foods, spat food out more often).
Wright et al., 2000 - Sample 1 (also reported in Wright & Birks, 2000)	125	15-18 mths	M=69 F=56	UK	FTT=97 Non-FTT=28	Feeding behaviour questionnaire 3-day diet diary	CS-CC CS-A	Significant difference (FTT rated more often as 'variable eater'; non-FTT rated more often as 'hungry'; FTT rated as liking most foods less often than non-FTT; FTT). Significant association (lower BMI & lower energy intake; greater severity of FTT & lower energy intake.

Author & year[a]	Sample Characteristics					Measure	Design[e]	Findings
	n	Age	Sex[b]	Nationality / Ethnicity[c]	FTT status[d]			
Wright et al., 2000 - Sample 2	89	15-17 mths	M=41 F=48	UK	FTT=44 Non-FTT=45	Feeding behaviour questionnaire 3-day diet diary	CS-CC	No significant group difference (energy intake; 'enjoyment of mealtimes', although a trend for less enjoyment). Significant difference (FTT ate fewer foods; FTT less 'hungry' at mealtimes).

[a] Only the abstract was available so much of the information was not accessible.
[b] -[NR], not reported.
[c] W, 'white'.
[d] FTT, 'failure to thrive'; non-FTT, 'non failure to thrive'.
[e] CS-CC, cross-sectional case-control design using child FTT/ non-FTT categorisation; CS-A, cross-sectional design using continual association between child's BMI & eating behaviour.

Appendix 2. Tables summarising the literature on the familiality and heritability of appetitive traits

Appendix 2.1. Famiality of appetitive traits (family studies)

Author & Year[a]	*n* (design)	Age	Sex	Nationality / Ethnicity[b]	Measure[c]	Findings	
Provencher et al., 2005	202 adult families (143 fathers; 189 mothers; 139 sons; 213 daughters)	>18 yrs	M=282 F=402	French Canadian	TFEQ	**Familiality** Restraint: 6% Disinhibition: 18% Hunger: 28%	**Unique environmental influence** Restraint: 94% Disinhibition: 82% Hunger: 72%
Steinle et al., 2002	28 adult families (436 parent/ offspring pairs; 1326 sibling pairs; 1342 avuncular pairs; 1311first cousin pairs)	46 yrs (mean)	M=286 F=338	US Old Order Amish	TFEQ	**Familiality** Restraint: 28% Disinhibition: 40% Hunger: 23%	**Unique environmental influence** Restraint: 72% Disinhibition: 60% Hunger: 77%
Agras et al., 1988	29 young families (parents & children)	18-months (mean for children)	M&F	US	Observed eating behaviour in laboratory (meal duration, eating speed, intake)	Significant parental association between caloric intake (0.37) and moderate but not significant association for eating speed (0.34), although very small sample size. No association for meal duration (0.0). Significant parent-child associations for eating speed (0.38), meal duration (0.31) and intake (0.35).	
Cutting et al., 1999	75 young families (75 children; 84 parents – 47 mothers, 37 fathers)	3-6 yrs (children); 39 yrs (mean for parents)	M=77 F=82 (children)	US W=88% B=3% H=3% Other=6%	TFEQ – 'disinhibition' (parents); EAH-lab (children)	Significant association between mothers' 'disinhibition' and daughters' 'eating in the absence of hunger' (0.41). Similar trend for mothers and sons but not significant (0.21). No significant association between fathers and children.	
Jahnke & Warschburger, 2008	142 young families (mothers & children)	3-6 yrs (children) 35 yrs (mean for mothers)	M=91 F=51 (children)	German	DEBQ & DEBQ-C ('emotional eating' & 'external eating') & CEBQ-FR & eating speed (1 item)	Significant association between mother's 'external eating' and daughters' and sons' 'external eating', but not with children's 'food responsiveness' or eating speed. Significant association between mother's 'emotional eating' and son's 'emotional eating' (0.29) but not daughter's 'emotional eating', although the sample was underpowered to detect the small association (0.14).	
Johnson & Birch, 1994	77 young families (parents & children)	3-5 yrs (children)	M=31 F=46 (children)	US W=82% Non-W=18%	TFEQ – 'disinhibition' (parents); preload (children)	Significant association between parental 'disinhibition' and children's compensation ability – higher disinhibition and lower compensation ability (-0.35).	

Author & Year[a]	*n* (design)	Age	Sex	Nationality / Ethnicity[b]	Measure[c]	Findings	
Faith et al., 2004	32 sibling pairs (children)	3-7 yrs	M=28 F=36	US W=25% B=40% H=25% Other=10%	High/low energy preload & ad libitum meal; (compensation ability; total energy intake;macronutrient intake	Significant sibling correlation for total energy intake, % fat intake, % carbohydrate intake, % protein intake. No significant sibling correlation for compensation ability.	
Fisher et al., 2007	300 young families 801 siblings (children)	4-19 yrs	M=406 F=395	US H	Ad libitum meal & EAH	**Familiality** Meal energy intake: 52% EAH: 51%	**Unique environmental influence** Meal energy intake: 48% EAH: 49%
Falciglia et al., 2004	33 young families (parents & children)	9-11 yrs (children) 33-49 yrs (parents)	M=15 F=18 (children)	US W=88% B=9% Other=3%	Food Neophobia Scale (parent-report questionnaire)	Significant associations between parental and child neophobia (0.34).	
Koivisto & Sjoden, 1996	57 young families (75 sons; 55 daughters; 57 mothers; 44 fathers)	2-17 yrs (children)	M=119 F=112	Swedish	Food Neophobia Scale & General Neophobia Scale (self-report, interview or parent-report questionnaire depending on age)	Significant associations between mother and daughter for Food Neophobia Scale (0.52), but no significant parent-child correlation for General Neophobia Scale. Mother and father were not significantly correlated for either scale.	
Koivisto & Sjoden, 1997	722 young families (722 parents & children)	7-17 yrs (children)	M&F	Swedish	Food Neophobia Scale & General Neophobia Scale (self-report or parent-report questionnaire)	Significant associations between parents and chilcren for the Food Neophobia Scale & General Neophobia Scale at a number of ages. Also smaller, but significant correlations between mothers and fathers.	
Pliner & Loewen, 1997	81 young families (sibling pairs & mothers)	5-11 yrs (children)	M=78 F=84	Canadian	Food Neophobia Scale (parent report questionnaire)	Significant associations between mothers and children for the Food Neophobia Scale, but no significant correlations between siblings.	
Logue et al., 1988	77 families (adults & children - 77 probands, 42 siblings, 68 mothers, 54 fathers)	15 yrs (mean for children) 42 yrs (mean for parents)	M=105 F=136	US	Food preferences questionnaire – 55 foods classified into 10 groups	Significant familial associations for 9 of the factors and correlations were moderate (0.24-0.50), but no significant correlations for fruit. Most correlations were between spouses and female family members.	

Author & Year[a]	*n* (design)	Age	Sex	Nationality / Ethnicity[b]	Measure[c]	Findings
Pliner & Pelchat, 1986[a]	55 families (parents & children)	-	M&F	Canadian	Food preferences questionnaire (mother-report)	Significant correlations between all family members, but especially large in siblings.
Rozin, 1991	118 families (parents & children)	19 yrs (mean for children) 46 yrs (mean for mother) 49 yrs (mean for father)	M=46 F=72 (children)	US W=90% Non-W=10%	Food preferences questionnaire (self-report) – 11 foods assessed individually	Parent-child correlations were the same as parent-parent correlations. The highest correlations were for strong tastes (hot sauces and black coffee).

[a] Only the abstract was available so much of the information was not accessible.
[b] W, 'white'; non-W, 'non-White'; H, 'Hispanic'; B, 'Black'.
[c] TFEQ, Three Factor Eating Questionnaire; DEBQ, Dutch Eating Behaviour Questionnaire; DEBQ-C, Dutch Eating Behaviour Questionnaire, child-report version; EAH, 'eating in the absence of hunger' (EAH-lab, experiment took place in a laboratory setting); CEBQ-FR, Child Eating Behaviour Questionnaire, 'food responsiveness' scale.

Appendix 2.2. Heritability of appetitive traits (twin studies)

Author & Year[a]	*n* (design)[b]	Age	Sex	Nationality / Ethnicity[c]	Measure[d]	Findings		
						Genetic influences	Shared environmental influences	Unique environmental influences
de Castro & Lilenfeld, 2005	149 twin pairs MZ=39 DZ=110	44 yrs (mean)	M=160 F=138	US	TFEQ	Restraint: 44% Disinhibition: 0% Hunger: 24%	Restraint: 0% Disinhibition: 40% Hunger: 0%	Restraint: 56% Disinhibition: 60% Hunger: 76%
Keskitalo et al., 2008	641 twin pairs MZs=314 DZs=327	17-82 yrs	M=216 F=1066	British & Finnish	TFEQ-18 (shorter version)	Restraint: 26-63% Disinhibition: 45-69% Emotional eating: 9-45%	Restraint: 0% Disinhibition: 0% Emotional eating: 0%	Restraint:37-74% Disinhibition: 31-55% Emotional eating: 55-91%
Neale et al., 2003	210 twin pairs (& 152 twins with no co-twin) MZs=129 DZs=81	Adults	F	US	TFEQ (modified 36 item version)	Restraint: 0% Disinhibition: 45% Hunger: 8%	Restraint: 31% Disinhibition: 0% Hunger: 16%	Restraint: 69% Disinhibition: 55% Hunger: 76%
Tholin et al., 2005	782 twin pairs MZs=456 DZs=326	23-29 yrs	M	Swedish	TFEQ-R21 (revised version)	Restraint: 59% Disinhibition: 45% Emotional eating: 60%	Restraint: 0% Disinhibition: 0% Emotional eating: 0%	Restraint: 41% Disinhibition:55% Emotional eating: 40%
Schur et al., 2009	720 twin pairs MZs=438 DZs=282	19-81 yrs	M=509 F=931	US W=90% Non-W=10%	Restraint Scale	43%	0%	57%
Sung et al., 2010	583 families (691 twin pairs & 1010 other family members including P-O pairs & S-S pairs) MZs=443 DZs=248	20-65 yrs	M=816 F=1328	Korean	DEBQ	Restraint: 31% Emotional eating: 25% External eating: 25%	Restraint: 0% Emotional eating: 0% External eating: 0%	Restraint: 69% Emotional eating: 75% External eating: 75%
Carnell et al., 2008	5435 twin pairs	8-11 yrs	M=5278 F=5592	UK W=93% non-W=7%	CEBQ-SR/SE & CEBQ-EF	CEBQ-SR/SE: 63% CEBQ-EF: 75%	CEBQ-SR/SE: 21% CEBQ-EF: 10%	CEBQ-SR/SE: 16% CEBQ-EF: 15%

Author & Year[a]	*n* (design) [b]	Age	Sex	Nationality / Ethnicity[c]	Measure[d]	Findings		
						Genetic influences	Shared environmental influences	Unique environmental influences
Llewellyn et al., 2008	127 twin pairs MZs=63 DZs=64	8-11 yrs	M=102 F=152	UK W	Eating speed observed at home (bites / minute)	62%	0%	38%
Cooke et al., 2007	5390 twin pairs MZ=1913 DZ=3477	8-11 yrs	M=5237 F=5543	UK W=93% non-W=7%	Child Food Neophobia Scale (parent-report)	78%	0%	22%
Knaapila et al., 2007	28 families (70 P-O pairs; 145 S-S pairs; 47 C-C pairs) 468 twin pairs MZs=211 DZs=257	19-74 yrs	M=50 F=1041 (all twins)	Finnish families British twins	Food Neophobia Scale (self-report)	British: 67% Finnish: 69%	British: 0% Finnish: 0%	British:33% Finnish: 31%
Knaapila et al., 2010	515 twin pairs (& 145 twin with no co-twin data) MZs=206 DZs=309	20-25 yrs	M=532 F=643	Finnish twins	Food Neophobia Scale (self-report)	Male; Female 0%; 61%	Male; Female 45%; 0%	Male; Female 55%; 39%
Breen et al., 2006	214 twin pairs 103 MZ 111 DZ	4-5 yrs	M=205 F=223	UK	Food preferences questionnaire (parent-report)	Vegetables: 37% Desserts: 20% Meat & fish: 78% Fruit: 51%	Vegetables: 51% Desserts: 64% Meat & fish: 12% Fruit: 32%	Vegetables: 13% Desserts: 16% Meat & fish: 10% Fruit: 17%
Falciglia & Norton, 1994[a]	35 twin pairs MZs=14 DZs=21	9-18 yrs	-	US	Food preferences questionnaire (self-report) & taste test	Significant genetic influences on orange juice, broccoli, cottage cheese, chicken, sweetened cereal and hamburger (MZ correlation significantly higher than DZ correlation).		

Author & Year[a]	*n* (design) [b]	Age	Sex	Nationality / Ethnicity[c]	Measure[d]	Findings		
						Genetic influences	**Shared environmental influences**	**Unique environmental influences**
Faust, 1974	96 twin pairs MZs=48 DZs=48	17-40 yrs	M=96 F=96	British	Food likes & dislikes questionnaire – 7 different food groups & 7 individual foods	No difference between MZ and DZ correlations for any foods except spicy food.		
Keskitalo et al., 2008	641 twin pairs MZs=314 DZs=327	17-82 yrs	M=216 F=1066	British & Finnish	Liking & use of sweet & fatty foods, and salty & fatty foods (food preferences questionnaire)	Liking & use of fatty foods: 45% Some sex differences in estimates	Liking & use of fatty foods: 0%	Liking & use of fatty foods: 55%
Krondl et al., 1983	23 twin pairs MZs=13 DZs=10	Adults	F	Canadian	Food preferences questionnaire – 24 individual foods	One third of the foods showed a significant genetic component, including bacon, unsweetened grapefruit, apple and orange juices, strawberries, green beans and broccoli.		
Rozin & Millman, 1987	72 twin pairs MZs=38 DZs=34	17-26 yrs	M=62 F=82	US W=50% B=50%	Food preferences questionnaire - 13 individual foods & spiciness	Significant twin correlations across the board for most food preferences but MZ correlations not significantly higher than DZ, indicating shared environmental influences. But significantly higher MZ correlation for spiciness.		

[a] Only the abstract was available so much of the information was not accessible.
[b] MZ, monozygotic; DZ, dizygotic.
[c] W, 'white'; non-W, 'non-White'; B, 'Black'.
[d] TFEQ, Three Factor Eating Questionnaire; DEBQ, Dutch Eating Behaviour Questionnaire; CEBQ, Child Eating Behaviour Questionnaire (CEBQ-SR/SE, 'satiety responsiveness' and 'slowness in eating' scales combined; CEBQ-EF, 'enjoyment of food' scale).

Appendix 2.3. Genetic and environmental correlations between different appetitive traits, and appetitive traits and adiposity

Author & Year	Phenotypes	Correlations			Proportion of phenotypic correlation explained by:	
		Phenotypic	Genetic	Unique Environmental	Common genetic factors[a]	Common environmental factors[a]
(Fisher et al., 2007)	Dinner intake & eating in the absence of hunger	0.23	0.22 (ns)	0.22 (ns)	nr	nr
	Dinner intake & BMI	0.63	0.58	0.70	nr	nr
	Dinner intake & fat mass	0.65	0.65	0.67	nr	nr
	Dinner intake & fat free mass	0.61	0.40	0.78	nr	nr
	Eating in the absence of hunger & BMI	0.11	0.00 (ns)	0.23	nr	nr
	Eating in the absence of hunger & fat mass	0.11	0.00 (ns)	0.25	nr	nr
	Eating in the absence of hunger & fat free mass	0.16	0.21 (ns)	0.18 (ns)	nr	nr
(Keskitalo et al., 2008)	Cognitive restraint & uncontrolled eating	0.06	0.20	0	100%	0%
	Cognitive restraint & emotional eating	0.24	0.42	0	100%	0%
	Emotional eating & uncontrolled eating	0.56	0.75	0.50	58%	42%
	Cognitive restraint & BMI	0.13	0.16	0	100%	0%
	Uncontrolled eating & BMI	0.14	0.29	0.13	81%	19%
	Emotional eating & BMI	0.31	0.51	0.16	81%	19%
(Neale et al., 2003)	Disinhibition & hunger	0.79	0.39	0.77	39%	61%

[a]nr, not reported.

Appendix 3. Gemini study materials

Appendix 3.1. Gemini first contact letter

gemini
health and development in twins

DEPARTMENT OF EPIDEMIOLOGY & PUBLIC HEALTH
HEALTH BEHAVIOUR RESEARCH CENTRE

UCL

WELCOME TO GEMINI

Thank you for showing interest in our exciting new study: Gemini - which we are launching at UCL (University College London). You recently send a reply card back to the Office of National Statistics stating your interest in participating in this research. We are pleased to welcome you into Gemini.

Within the next few weeks, we are sending out the first questionnaires. We can also offer a web-based version if that is easy for you.

If you would like to fill out our web-based questionnaires we can send you the link by email. Please send us an email with your full name and postcode at Gemini@public-health.ucl.ac.uk indicating that you would like to receive the Gemini link and fill out questionnaires online.

If you prefer paper copies of questionnaires then you do not need to contact us; we will send you paper questionnaires and a pre-paid envelope (no stamp required) for easy return within the next few weeks.

If you have any queries about the study, please contact the Gemini team (Rebecca, Ellen, Clare or Laura) at 020 7679 6643 or send us an email at Gemini@public-health.ucl.ac.uk .

Kind regards,

Jane Wardle

Professor Jane Wardle

Appendix 3.2. Gemini baseline questionnaire letter

gemini
health and development in twins

DEPARTMENT OF EPIDEMIOLOGY & PUBLIC HEALTH
HEALTH BEHAVIOUR RESEARCH CENTRE

UCL

Dear <<Name Mother>> Family ID Number: GEM<<FAM ID>>

Thank you for indicating your interest in our exciting new study: Gemini – health and development in twins. You recently sent a reply card back to the Office of National Statistics stating that you would like to participate in this research. We are pleased to welcome you into Gemini.

Together with this letter you will find two copies of the consent form. To confirm your interest in Gemini, we would like to ask you to initial all the boxes on the consent form and sign them, and send one copy back to us together with the questionnaires and keep one copy for yourself.

Questionnaires
We have divided our questions in to two booklets for your convenience. The questionnaires are about your twins' growth, eating and activity habits and your views on feeding. The questionnaires also include some questions about various aspects of your home and family life. The information you provide will remain completely confidential. The questionnaires shouldn't take very long to complete and are designed to allow you to answer questions section by section at your own leisure.

Can I fill out the questionnaires online?
Yes, the questionnaires are available on the internet. If you would like to fill out our web-based questionnaires instead of the paper-based ones, please go to the following webpage, and follow the instructions: http://www.attitudestohealth.co.uk/gemini/
Please note that questions are divided into two parts just like the paper-based questionnaires. Each part needs to be completed in one go; although it is possible to take short breaks (15 minutes) as long as you leave the web browser open. Once you have started part one you will not be able to save it and come back to it later. However, after completing part one you can come back and complete part two (in one go) at your convenience.

What do I need to send back?
After you have signed the consent form and answered all of the questions in the two booklets, you can send us the consent form and both booklets in the freepost envelope (no stamps required). If you completed the questionnaires online, then just send the signed consent form in the freepost envelope to: Gemini, Health Behaviour Research Centre, UCL, 2-16 Torrington Place, London WC1E 6BT.

When will I be contacted again?
We would like to contact you again when the twins are about 15 months old. You do not have to let us know now whether or not you would like to continue to participate. We will confirm this with you the next time we contact you.

If you have any queries about the study, please contact the Gemini team (Rebecca, Ellen, Clare or Laura) at 020 7679 6643 or send us an email at Gemini@public-health.ucl.ac.uk.

Kind regards,

Jane Wardle

Professor Jane Wardle

Appendix 3.3. Gemini consent form

gemini
health and development in twins

DEPARTMENT OF EPIDEMIOLOGY & PUBLIC HEALTH
HEALTH BEHAVIOUR RESEARCH CENTRE

UCL

CONSENT FORM

Title of Project: Gemini – health and development in twins

Study Reference: 07/H0714/116 Family ID number:

Name of Researchers: *Professor Jane Wardle, Dr. Ellen van Jaarsveld*

	Please initial box
1. I confirm that I have read and understood the information leaflet for the above study which I received with the invitation letter. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2. I confirm that I have had sufficient time to consider whether or not I want to be included in the study.	☐
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my legal rights being affected.	☐
4. I consent to the processing of my personal information for the purposes of this study, and that it will not be used for any other purpose. I understand that such information will be treated as strictly confidential and handled in accordance with the provisions of the Data Protection Act 1998.	☐
5. I agree to be contacted in the future by the Gemini team who would like to invite me to participate in follow-up studies.	☐
6. I understand that the information I have submitted will be published as a report. Confidentiality and anonymity will be maintained and it will not be possible to identify me from any publications.	☐
7. I understand that some study documents may be looked at by responsible representatives from the Research & Development Unit, UCL to ensure that the study is being conducted properly. My identity will be protected at all times.	☐
8. I agree to take part in the above study.	☐

Name of Participant	**Date**	**Signature**

Name of Researcher: Jane Wardle **Date**: 23 / 07 / 2008 **Signature**: Jane Wardle

When completed: Please keep 1 copy for your own records; and send 1 copy back to:
Gemini, Health Behaviour Research Centre, UCL, 2-16 Torrington Place, London, WC1E 6BT

Appendix 3.4. Gemini participant information leaflet

Do I have to take part?

It is up to you to decide whether to take part. If you choose not to participate it will involve no penalty or loss of benefits to which you are otherwise entitled. If you decide to take part you have this information sheet to keep and you will be asked to sign a consent form. If you decide to take part now, you are still free to withdraw at any time without giving a reason.

How can I take part in Gemini?

If you are interested in taking part in Gemini, please fill out the reply slip and return it in the enclosed envelope to ONS.

The more families that agree to take part, the more valuable the study will be. The team looks forward to your response.

Who has approved this study?

This study has been reviewed and approved by Cancer Research UK and UCL/UCLH Research Ethics Committee.

Who is running the study?

Gemini is being conducted by the Health Behaviour Research Centre, which is part of University College London. The study has been financed by Cancer Research UK, because of their interest in healthy food choices.

DEPARTMENT OF EPIDEMIOLOGY & PUBLIC HEALTH
HEALTH BEHAVIOUR RESEARCH CENTRE
UCL

For further information, please contact us:

T 020 7679 6643
E Gemini@public-health.ucl.ac.uk

Professor Jane Wardle - Principal Investigator
Dr Ellen van Jaarsveld - Study Coordinator
Ms Clare Llewellyn - Researcher
Dr Laura Johnson - Researcher
Ms Rebecca Marlow - Administrative Assistant

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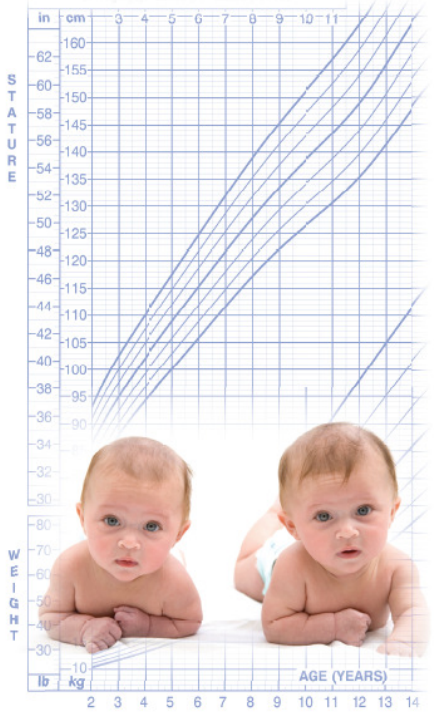

Information leaflet

gemini

health and development in twins

We are inviting you to join our new research project - Gemini. We are asking you because you have had twins – congratulations! Twins are very special to their parents. They are also very interesting to researchers because studying them can tell us about how genes and environments work together.

You should only take part if you want to; choosing not to participate will not disadvantage you in any way. Before you decide, you should read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or you want more information.

What is Gemini?

Gemini is a new national study following 2,400 parents and their twins through their first five years. We shall be interested in the twins' health, eating habits and activity with a main focus on appetite and weight gain. We are also interested in parents' attitudes and aspects of the home situation.

What does taking part involve?

Each year, we would like to send you questionnaires about your twins' development. A freepost envelope will be enclosed for easy return of the questionnaires. We can also offer a web-based version if that is easy for you. The information you provide will remain completely confidential. The questionnaire should take no longer than 90 minutes to complete.

Selecting families

The General Register Office (part of the Office for National Statistics or ONS) have identified your name as the mother of twins from their Birth Registration database. They are helping the Gemini study by contacting families with newborn twins. Names and addresses will only be made available to Gemini by ONS when parents have agreed to participate. Only families in England and Wales with registered twin births in 2007-2008 will be contacted.

Anticipated benefits to you and your family

Being part of Gemini should be interesting and fun! Families involved in an earlier study in which twins were followed from age 4 to 11 years have told us they enjoyed the experience.

One parent said, "I thought I would be too busy to do the study but I found it was an opportunity to stop and reflect about my twins and our life together as a family. It was time well spent!"

"I think it's great that my twins are part of the study and they think they're pretty special for being selected" said another parent.

"I've enjoyed reading the newsletter and read about some twins being very similar and others being totally different" said another parent.

Will the information I give remain confidential?

Yes. The privacy of the families and twins taking part in the study is strictly protected. All information collected about you and your child during the course of the research will be kept strictly confidential. The research team will not pass on your personal details to anyone else. Professional standards of confidentiality will be adhered to and all data will be collected and stored in accordance with the Data Protection Act 1998.

How will the information be used?

The information collected in the study will help us learn more about children's growth and health. It could also help the government to plan policies and services that benefit families and children. We will report our findings in academic and health-related journals and present them to relevant health professionals at meetings and conferences. You will not be identified in any reports or publications arising from the study.

Appendix 3.5. Gemini baseline questionnaire

Family ID Number ☐☐☐☐☐☐

WELCOME TO

gemini

health and development in twins

Booklet 1 - You and Your Family

Health Behaviour Research Centre
Department of Epidemiology & Public Health
UCL
2-16 Torrington Place
London, WC1E 6BT
Gemini@public-health.ucl.ac.uk

HOW TO FILL IN THIS BOOKLET

Thank you for agreeing to fill out this booklet. Before you start, here is a bit of guidance:

- We realise that parents of twins are very busy! We are especially grateful.
- We know the questionnaire is quite long, but please try to answer *all* the questions you are asked. This will help us to get a full picture of you and your twins' circumstances.
- Please be as honest as you can when answering our questions. We want to know what you really think. Everything you tell us will be kept strictly confidential.
- This may sound obvious, but please write as clearly as possible. This will help us use all the valuable information you have provided.

Here is an example of how a question *could* be answered.

Most of the questions in this booklet will ask you to tick a box next to the answer that is most suitable. Some will also ask you to describe this answer in more detail, for example:

A1. **Do you think your twins are identical or non-identical?** Identical ☑ Non-identical ☐

Why do you think this? ...The twins shared the same sac and placenta...

A2. **As your twins grow older, do you have more time for yourself?** Yes ☑ No ☐

THIS QUESTIONNAIRE IS TO BE COMPLETED BY THE MOTHER OF THE TWINS.
IF YOU ARE NOT THE MOTHER, PLEASE CONTACT US AND WE WILL SEND YOU THE APPROPRIATE QUESTIONNAIRE

THANK YOU FOR YOUR TIME AND ASSISTANCE IN FILLING OUT THIS BOOKLET

YOUR TWINS

A1. Are you the primary caregiver of the twins? Yes ☐ No ☐

A2. What is your first born twin's name? _____

Is your first born twin a boy or a girl? Boy ☐ Girl ☐

What is his/her date of birth? ____ / ____ / _____
DD MM YYYY

A3. What is your second born twin's name? _____

Is your second born twin a boy or a girl? Boy ☐ Girl ☐

The next few questions are all about whether your twins are identical or non-identical. This section needs to be completed only if you have same sex twins (please note: non-identical twins are often called fraternal twins)

If your twins are opposite sex, please go straight to B1 on page 6

A4. Have you ever been told by a health professional (e.g. doctor, nurse, consultant) that your twins are identical or non-identical?

Yes, identical ☐ Yes, non-identical ☐ No ☐

If YES, why did they think this? _____

_____

A5. Do you think your twins are identical or non- identical?

Identical ☐ Non-identical ☐

Why do you think this is?

_____

A6. **As your twins have grown older, has the likeness between them:**

Become less ☐ Remained the same ☐ Become more ☐

A7. **When looking at the twins:**

	None	Only slight difference	Clear difference
Are there differences in the shade of your twins' hair?	☐	☐	☐
Are there differences in the texture of your twins' hair (fine or coarse, straight or curly etc)?	☐	☐	☐
Are there differences in the colour of your twins' eyes?	☐	☐	☐
Are there differences in the shape of your twins' ear lobes?	☐	☐	☐

A8. **Have either of your twins' teeth begun to come through?** Yes ☐ No ☐

If yes, was it at about the same time?

Yes, the twins had matching teeth on the same side come through within a few days of each other ☐

Yes, the twins had matching teeth on opposite sides come through within a few days of each other ☐

Yes, the twins had different teeth come through within a few days of each other ☐

No, the twins' first teeth did not come through within a few days of each other ☐

A9. **Do you know your twins' ABO blood group and Rhesus (Rh) factors?**

Yes ☐ No ☐

If YES, what are they? (please tick a blood group and rhesus factor for each twin)

	Blood group:				Rhesus factor:	
	A	B	AB	O	Rh+	Rh-
1st born	☐	☐	☐	☐	☐	☐
2nd born	☐	☐	☐	☐	☐	☐

A10. **When looking at a new photograph of your twins, can you tell them apart (without looking at their clothes or using any other clues)?**

Yes, easily	Yes, but it is hard sometimes	No, I often confuse them in photographs
☐	☐	☐

A11. **Do any of the following people ever mistake your twins for each other?**

	Yes, often	Yes, sometimes	Rarely or never	Not applicable
Your partner / husband	☐	☐	☐	☐
Older brothers or sisters	☐	☐	☐	☐
Other relatives	☐	☐	☐	☐
Babysitter or day carer	☐	☐	☐	☐
Close friends	☐	☐	☐	☐
Casual friends	☐	☐	☐	☐
People meeting the twins for the first time	☐	☐	☐	☐

A12. **If the twins are ever mistaken for one another, does this ever happen when they are together?**

Yes, often	Yes, sometimes	No, almost never	They are not mistaken for one another
☐	☐	☐	☐

A13. **Would you say that your twins:**

Are as physically alike as "two peas in a pod" (virtually the same) ☐

Are as physically alike as brothers and sisters are ☐

Do not look very much alike at all ☐

ABOUT YOU

B1. **What is your date of birth?** _____ / _____ / _____ (DD / MM / YYYY)

B2. **In general, would you say your own health is:**

Excellent ☐ Very good ☐ Good ☐ Fair ☐ Poor ☐

B3. **About how tall are you?**

_____ centimetres (cms) OR _____ feet (ft) and _____ inches

B4. **About how much do you weigh?** If possible, use weighing scales for current weights, otherwise please give estimates

_____ kilograms (kgs) OR _____ stones (st) and _____ pounds (lbs)

B5. **Given your age and height, would you say that you are:**

Very underweight ☐ Slightly underweight ☐ About the right weight ☐ Slightly overweight ☐ Very overweight ☐

B6. **Do you have any educational qualifications?** (please tick all that apply or equivalents)

No qualifications ☐ CSE, GCSE or 'O' Level ☐ Vocational qualification (GNVQ, BTEC) ☐ 'A' or 'AS' level ☐ Higher National Certificate (HNC) or Diploma (HND) ☐ Undergraduate degree ☐ Postgraduate qualification (Masters, PhD) ☐

Other, please describe: _____

B7. **Do you currently have a job?**

On maternity leave ☐ Yes, full-time ☐ Yes, part-time ☐ No ☐ Stay at home to look after the children ☐

If NO, or stay at home to look after children please go straight to B9 on page 7

B8. **What is your FULL job title?** (please describe) _____

Do you need any special qualifications for your job?

Yes ☐ No ☐ Unsure ☐

If YES, please describe:

_____

6

B9. What is your ethnic group? Tick the appropriate box to indicate your cultural background

White	Black	Asian	Mixed	Chinese or any other
White British ☐	Caribbean ☐	Indian ☐	White and Black Caribbean ☐	Chinese ☐
White Irish ☐	African ☐	Pakistani ☐	White and Black African ☐	
		Bangladeshi ☐	White and Asian ☐	
Other White background ☐ (please specify)	Other Black background ☐ (please specify)	Other Asian background ☐ (please specify)	Other Mixed background ☐ (please specify)	Any other (please specify) ☐
_____		_____		_____

B10. Do you smoke cigarettes at all nowadays? Yes ☐ No ☐

If Yes, how many cigarettes a day do you usually smoke? ____ cigarettes per day

B11. Do you usually participate in the following activities? If so, how many times per week and for how long? (Write 0 if you do not participate in any activity)

Strenuous exercise (heart beats rapidly)
i.e. running, jogging, hockey, football, squash, vigorous swimming, vigorous cycling ____ times per week ____ minutes per session

Moderate exercise (not exhausting)
i.e. fast walking, tennis, easy cycling, badminton, easy swimming, dancing ____ times per week ____ minutes per session

Mild exercise (minimal effort)
i.e. yoga, fishing from river bank, bowling, golf, easy walking ____ times per week ____ minutes per session

B12. In the last week about how many servings of ..... did you eat?

	Less than 1 per week	1 per week	2-4 per week	5-6 per week	1 per day	2 per day	3 per day	4 or more per day
VEGETABLES (excluding potatoes)	☐	☐	☐	☐	☐	☐	☐	☐
FRUIT (fresh, frozen or canned)	☐	☐	☐	☐	☐	☐	☐	☐

B13. What is your marital status?

Married or cohabiting ☐ Divorced ☐ Widowed ☐ Separated ☐ Single ☐

If you are not married or cohabiting, please go to straight to D1 on page 10

ABOUT THE PERSON YOU LIVE WITH
(i.e. your husband or partner)

C1. What is your partner's relationship to the twins?

Natural father of the twins ☐ Legal guardian of the twins ☐ Other ☐

If other, please describe: _____

C2. What is your partner's date of birth? ____ / ____ / _____
DD MM YYYY

C3. About how tall is your partner?

_____ centimetres (cms) OR _____ feet (ft) and _____ inches

C4. About how much does your partner weigh? If possible, use weighing scales for current weights, otherwise please give estimates

_____ kilograms (kgs) OR _____ stones (st) and _____ pounds (lbs)

C5. Does your partner have any educational qualifications? (please tick all that apply or equivalents)

No qualifications	CSE, GCSE or 'O' Level	Vocational qualification (GNVQ, BTEC)	'A' or 'AS' level	Higher National Certificate (HNC) or Diploma (HND)	Undergraduate degree	Postgraduate qualification (Masters, PhD)
☐	☐	☐	☐	☐	☐	☐

Other, please describe: _____

C6. Does your partner currently have a job?

Yes, full-time	Yes, part-time	No	Stay at home to look after the children
☐	☐	☐	☐

If NO, or stay at home to look after children, please go straight to C8 on page 9

C7. What is your partner's FULL job title? (please describe) _____

Are there any special qualifications needed for their job?

Yes ☐ No ☐ Unsure ☐

If YES, please describe: _____

8

C8. **What is your partner's ethnic group?** Tick the appropriate box to indicate your partner's cultural background

White	Black	Asian	Mixed	Chinese or any other
White British ☐	Caribbean ☐	Indian ☐	White and Black Caribbean ☐	Chinese ☐
White Irish ☐	African ☐	Pakistani ☐	White and Black African ☐	
		Bangladeshi ☐	White and Asian ☐	
Other White background (please specify) ☐	Other Black background (please specify) ☐	Other Asian background (please specify) ☐	Other Mixed background (please specify) ☐	Any other (please specify) ☐
_____	_____	_____		_____

C9. **Does your partner smoke cigarettes at all nowadays?** Yes ☐ No ☐

If Yes, how many cigarettes a day does your partner usually smoke? ____ cigarettes per day

C10. **Does your partner usually participate in the following activities? If so, how many times per week and for how long?** (Write 0 if your partner does not participate in any activity)

Strenuous exercise (heart beats rapidly)
i.e. running, jogging, hockey, football, squash, vigorous swimming, vigorous cycling ____ times per week ____ minutes per session

Moderate exercise (not exhausting)
i.e. fast walking, tennis, easy cycling, badminton, easy swimming, dancing ____ times per week ____ minutes per session

Mild exercise (minimal effort)
i.e. yoga, fishing from river band, bowling, golf, easy walking ____ times per week ____ minutes per session

C11. **In the last week about how many servings of ..... did your partner eat?**

	Less than 1 per week	1 per week	2-4 per week	5-6 per week	1 per day	2 per day	3 per day	4 or more per day
VEGETABLES (excluding potatoes)	☐	☐	☐	☐	☐	☐	☐	☐
FRUIT (fresh, frozen or canned)	☐	☐	☐	☐	☐	☐	☐	☐

ABOUT OTHER CHILDREN IN THE HOME

D1. How many other children live in the home with your twins? (please write number)

_____ children

If there are no other children living in the home, please go straight to E1 on page 11

D2. Please tell us about all the children who live in the home with the twins:

Child's name	Date of birth	Sex		Does the child have the same mother as the twins?		Does the child have the same natural father as the twins?	
		Boy	Girl	Yes	No	Yes	No
_____	___/___/____ DD MM YYYY	☐	☐	☐	☐	☐	☐
_____	___/___/____ DD MM YYYY	☐	☐	☐	☐	☐	☐
_____	___/___/____ DD MM YYYY	☐	☐	☐	☐	☐	☐
_____	___/___/____ DD MM YYYY	☐	☐	☐	☐	☐	☐
_____	___/___/____ DD MM YYYY	☐	☐	☐	☐	☐	☐
_____	___/___/____ DD MM YYYY	☐	☐	☐	☐	☐	☐

If there are more than six other children or if there is anything else you would like to tell us about your family, please tell us in the open space below

YOUR PREGNANCY WITH THE TWINS

E1. **About how much weight did you gain during your pregnancy with the twins?**

_____ kilograms (kgs) OR ____ stones (st) and ____ pounds (lbs)

E2. **When you became pregnant with the twins, were you having any fertility treatment?** Yes ☐ No ☐

If YES, please describe: _____

E3. **Were you regularly taking any medicine whilst pregnant?** Yes ☐ No ☐

If YES, was this: (please tick all that apply) For first 3 months ☐ For middle 3 months ☐ For last 3 months ☐

Please describe the type of medication: _____

E4. **Did you smoke any cigarettes whilst pregnant?** Yes ☐ No ☐

If YES, was this: (please tick all that apply) For first 3 months ☐ For middle 3 months ☐ For last 3 months ☐

How many cigarettes a day did you smoke, on average? (write 0 if you smoked no cigarettes whilst pregnant) _____ cigarettes per day

E5. **Did you drink any alcohol whilst pregnant?** Yes ☐ No ☐

If YES, was this: (please tick all that apply) For first 3 months ☐ For middle 3 months ☐ For last 3 months ☐

How many units of alcohol did you drink per week, on average? (1 unit = 1 glass of wine, or 1 measure of spirits, or ½ a pint of beer) (write 0 if you drank no alcohol whilst pregnant) _____ units per week

E6. **Did you experience any severe stress during your pregnancy (e.g. bereavement, serious illness in the family or major money problems)?** Yes ☐ No ☐

If YES, please describe: _____

_____

E7. During your pregnancy did you experience any of the following:

	Yes	No	Unsure
Morning sickness	☐	☐	☐
High blood pressure (pregnancy induced / gestational)	☐	☐	☐
Diabetes (pregnancy induced / gestational)	☐	☐	☐
Toxaemia / pre-eclampsia	☐	☐	☐
Vaginal bleeding	☐	☐	☐
Anaemia / iron deficiency	☐	☐	☐
Rubella / German Measles	☐	☐	☐
Slow growth of baby / babies	☐	☐	☐
Other serious pregnancy related problem (please describe)	☐	☐	☐

_____

_____

E8. Did you experience any physical or mental health problem in the first 6 months after birth; and were any of those problems diagnosed by a doctor?

Yes, diagnosed by a doctor ☐ Yes, but not diagnosed by a doctor ☐ No ☐

If YES, please describe: _____

_____

E9. Have you ever been diagnosed with heart disease or diabetes, before or after your pregnancy?

	Yes	No	Unsure
Heart disease	☐	☐	☐
Diabetes (unrelated to pregnancy)	☐	☐	☐

E10. In their lives, have any family members ever been diagnosed with heart disease or diabetes?

	Father of twins	Brother or sister of twins	Your mother	Your father	Mother of the twins' father	Father of the twins' father	None
Heart disease	☐	☐	☐	☐	☐	☐	☐
Diabetes	☐	☐	☐	☐	☐	☐	☐

E11. In general, how would you describe the weights of your family members throughout their lives?

	Very underweight	Slightly underweight	About the right weight	Slightly overweight	Very overweight	Unsure
Father of the twins	☐	☐	☐	☐	☐	☐
Your mother	☐	☐	☐	☐	☐	☐
Your father	☐	☐	☐	☐	☐	☐
Mother of the twins' father	☐	☐	☐	☐	☐	☐
Father of the twins' father	☐	☐	☐	☐	☐	☐

THE TWINS' BIRTH

F1. How many weeks pregnant were you at the time of delivery? _____ weeks

F2. Was the birth by Caesarean section?

Yes	No	Unsure
☐	☐	☐

If YES, why? _____

_____

F3. Approximately how long was the gap between the births?

_____ hours OR _____ minutes

F4. Did transfusion between twins occur (twin to twin transfusion syndrome)?

Yes	No	Unsure
☐	☐	☐

F5. Did your babies get a blood transfusion soon after birth?

Yes	No	Unsure
☐	☐	☐

F6. Were there any other complications or concerns about either twin at birth?

	Yes	No	Unsure
1st born	☐	☐	☐
2nd born	☐	☐	☐

If Yes in 1st born, please describe: _____

_____

_____

_____

If Yes in 2nd born, please describe: _____

_____

_____

_____

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F7. **Did either of the twins have any special care after birth (e.g. incubators)?**

	Yes	No
1st born	☐	☐
2nd born	☐	☐

If Yes in 1st born, please describe: _____

_____

If Yes in 2nd born, please describe: _____

_____

F8. **If yes, how long did they stay in special care?**

1st born	_____ days	or	_____ weeks
2nd born	_____ days	or	_____ weeks

F9. **How long did the twins stay in hospital after birth?**

1st born	_____ days	or	_____ weeks
2nd born	_____ days	or	_____ weeks

F10. **Do either of your twins have:**

	Yes, 1st born	Yes, 2nd born	Neither
Physical problems (e.g. cleft lip, hole in the heart)	☐	☐	☐

If Yes in 1st born, please describe: _____

If Yes in 2nd born, please describe: _____

	Yes, 1st born	Yes, 2nd born	Neither
Genetic or chromosomal problems (e.g. Down's Syndrome, PKU)	☐	☐	☐

If Yes in 1st born, please describe: _____

If Yes in 2nd born, please describe: _____

	Yes, 1st born	Yes, 2nd born	Neither
Any other medical problem after birth	☐	☐	☐

If Yes in 1st born, please describe: _____

_____

If Yes in 2nd born, please describe: _____

_____

F11. **Sometimes it is difficult to start feeding due to birth-related complications or other medical problems. Straight after birth, did either of your twins experience any complications which made it difficult to start feeding?**

Yes, in 1st born ☐ Yes, in 2nd born ☐ No ☐

If Yes in 1st born, please describe: _____

_____

If Yes in 2nd born, please describe: _____

_____

F12. **Were there any other times when feeding your twins was difficult, e.g. due to illness of the twins, health problems of parent, changes in jobs or moving house.**

Yes, in 1st born ☐ Yes, in 2nd born ☐ No ☐

If Yes, please describe for each twin: (Use the back of the questionnaire if you need extra space)

Problem 1 in 1st born _____

_____

_____

At which ages did this influence your twins eating? __to__ weeks or __to__ months

Problem 2 in 1st born _____

_____

_____

At which ages did this influence your twins eating? __to__ weeks or __to__ months

Problem 1 in 2nd born _____

_____

_____

At which ages did this influence your twins eating? __to__ weeks or __to__ months

Problem 2 in 2nd born _____

_____

_____

At which ages did this influence your twins eating? __to__ weeks or __to__ months

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THE TWINS' ILLNESSES AND ACCIDENTS

F13. **About how many times have your babies seen the doctor due to illness or accidents since birth?**

	Number of visits
1st born	_____
2nd born	_____

F14. **Since birth, have your babies been admitted to hospital?**

	No	Yes, once	Yes, more than once (write number)
1st born	☐	☐	_____
2nd born	☐	☐	_____

F15. **Please briefly describe each hospital admission** (Use the back of the questionnaire if you need more space)

	Age of twin (months)	Number of hospital nights	Reason for admission:
1st born	_____	_____	_____
	_____	_____	_____
	_____	_____	_____
	_____	_____	_____
	_____	_____	_____
	_____	_____	_____
2nd born	_____	_____	_____
	_____	_____	_____
	_____	_____	_____
	_____	_____	_____
	_____	_____	_____
	_____	_____	_____

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SOME FINAL QUESTIONS ABOUT YOU AND YOUR FAMILY

G1. **What is the main language spoken in the home?**

English ☐ Other (please specify) _____

G2. **Altogether, how many adults live in the same house as the twins (including yourself)?**

One ☐ Two ☐ Three ☐ Four or more: _____ (please give number)

G3. **How many bedrooms does your household have, including bedsitting rooms and spare rooms?**

One ☐ Two ☐ Three ☐ Four or more: _____ (please give number)

G4. **How many cars or vans are normally available for use by you or any members of your household?**

None ☐ One ☐ Two ☐ Three or more: _____ (please give number)

G5. **Do you currently own or rent the accommodation you live in?**

Own without mortgage	Own with mortgage	Rent privately	Rent from local authority
☐	☐	☐	☐

G6. **Thinking of the income of the household as a whole, which category represents the total income of your whole household before deduction from income tax, National Insurance etc.**

Up to £15,000 per year	☐	Between £52,500 and £60,000 per year	☐
Between £15,000 and £22,500 per year	☐	Between £60,000 and £67,500 per year	☐
Between £22,500 and £30,000 per year	☐	Between £67,500 and £75,000 per year	☐
Between £30,000 and £37,500 per year	☐	Between £75,000 and £82,500 per year	☐
Between £37,500 and £45,000 per year	☐	Between £82,500 and £90,000 per year	☐
Between £45, 000 and £52,500 per year	☐	More than £90,000 per year	☐

G7. **Do you feel your family income is enough?**

More than enough ☐ Enough ☐ Not enough ☐

G8. **Please give the date on which you completed this booklet?** ___ / ____ / ____ day/month/year

Please continue with BOOKLET 2 to tell us more about your twins

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Thank you

for filling out this booklet.

PLEASE continue with BOOKLET 2 to tell us more about your twins

Space for any additional comments you would like to make :

Family ID Number ☐☐☐☐☐☐

WELCOME TO

gemini

health and development in twins

Booklet 2 - Your Twins

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HOW TO FILL IN THIS BOOKLET

Thank you for agreeing to fill out this booklet. Before you start, here is a bit of guidance:

- We realise that parents of twins are very busy! We are especially grateful.
- We know the questionnaire is quite long, but please try to answer *all* the questions you are asked. This will help us to get a full picture of you and your twins' circumstances.
- Please be as honest as you can when answering our questions. We want to know what you really think. Everything you tell us will be kept strictly confidential.
- This may sound obvious, but please write as clearly as possible. This will help us use all the valuable information you have provided.

Here is an example of how a question *could* be answered.

Most of the questions in this booklet will ask you to tick a box next to the answer that is most suitable. Some will also ask you to describe this answer in more detail, for example:

A1. Do you think your twins are identical or non-identical? Identical [√] Non-identical [ ]

Why do you think this? ...The twins shared the same sac and placenta...

A2. As your twins grow older, do you have more time for yourself? Yes [√] No [ ]

THIS QUESTIONNAIRE IS TO BE COMPLETED BY THE MOTHER OF THE TWINS.
IF YOU ARE NOT THE MOTHER, PLEASE CONTACT US AND WE WILL SEND YOU THE APPROPRIATE QUESTIONNAIRE

THANK YOU FOR YOUR TIME AND ASSISTANCE IN FILLING OUT THIS BOOKLET

YOUR TWINS' GROWTH

First we would like to learn a bit more detail about your twins' growth. This information may be in your child's health record (little red book) or you may have kept your own records.

A1. What were the lengths of the twins at birth and around 6 weeks?

	1st born		2nd born	
At birth	_____ cm	or _____ inches	_____ cm	or _____ inches
Around 6 weeks	_____ cm	or _____ inches	_____ cm	or _____ inches

A2. What were the head circumferences of the twins?

	1st born		2nd born	
At birth	_____ cm	or _____ inches	_____ cm	or _____ inches
Around 6 weeks	_____ cm	or _____ inches	_____ cm	or _____ inches

A3. What were the weights of the twins?

	1st born		2nd born	
At birth	_____ kg	or ____ lbs ___ oz	_____ kg	or ____ lbs ___ oz
Around 6 weeks	_____ kg	or ____ lbs ___ oz	_____ kg	or ____ lbs ___ oz

A4. Please add other weight measurements below together with the date they were taken. Use the back of the questionnaire if you need extra space. Alternatively you can send us a photocopy of the relevant pages from your twins' health records (little red book)

Date measured	1st born				2nd born				These measurements came from…		
	kg		lbs,	oz	Kg		lbs,	oz	Health professional / health record		Own measure-ments
___/___/____ DD MM YYYY	_____	or	____	____	____	or	____	____	☐	or	☐
___/___/____ DD MM YYYY	_____	or	____	____	____	or	____	____	☐	or	☐
___/___/____ DD MM YYYY	_____	or	____	____	____	or	____	____	☐	or	☐
___/___/____ DD MM YYYY	_____	or	____	____	____	or	____	____	☐	or	☐
___/___/____ DD MM YYYY	_____	or	____	____	____	or	____	____	☐	or	☐
___/___/____ DD MM YYYY	_____	or	____	____	____	or	____	____	☐	or	☐
___/___/____ DD MM YYYY	_____	or	____	____	____	or	____	____	☐	or	☐
___/___/____ DD MM YYYY	_____	or	____	____	____	or	____	____	☐	or	☐

Some parents worry about their babies being underweight or overweight for their age and sex. The following questions explore this in a bit more detail

A5. How would you describe your baby's weight <u>at the moment?</u>

	Very underweight	Slightly underweight	About the right weight	Slightly overweight	Very overweight
1st born	☐	☐	☐	☐	☐
2nd born	☐	☐	☐	☐	☐

A6. Have you ever been concerned that your baby <u>wasn't gaining enough weight</u>? (tick <u>all</u> that apply)

	Yes	No
1st born	☐	☐
2nd born	☐	☐

If No, please go straight to A8 on page 5

If yes, how old was your baby when you were concerned?

	0 - 3 months	4-6 months	7-9 months	10-12 months	Older than 1 year
1st born	☐	☐	☐	☐	☐
2nd born	☐	☐	☐	☐	☐

A7. How concerned are you that your baby is <u>underweight at the moment</u>?

	Not concerned	Somewhat concerned	Very concerned
1st born	☐	☐	☐
2nd born	☐	☐	☐

If you are concerned about either baby being <u>underweight</u>, why is this? (please tick the most important reasons)

	1st born	2nd born
The health visitor/doctor advised me my baby is not gaining enough weight	☐	☐
Low centile on growth chart	☐	☐
My baby doesn't look as big as other babies of the same age and sex	☐	☐
My baby lost weight recently	☐	☐
My baby has always had a low weight	☐	☐
My baby is not feeding well	☐	☐
Family member(s) think my baby is not heavy enough. If so, who?_____	☐	☐
Other reason. If so, what? _____	☐	☐

A8. **Have you ever been concerned that your baby was gaining too much weight?**

	Yes	No
1st born	☐	☐
2nd born	☐	☐

If No, please go straight to B1 on page 6

If yes, how old was your baby when you were concerned?

	0 - 3 months	4-6 months	7-9 months	10-12 months	Older than 1 year
1st born	☐	☐	☐	☐	☐
2nd born	☐	☐	☐	☐	☐

A9. **How concerned are you that your baby is overweight at the moment?**

	Not concerned	Somewhat concerned	Very concerned
1st born	☐	☐	☐
2nd born	☐	☐	☐

If you are concerned about either baby being overweight, why is this? (please tick the most important reasons)

	1st born	2nd born
The health visitor/doctor advised me my baby is gaining too much weight	☐	☐
High centile on growth chart	☐	☐
My baby looks bigger than other babies of the same age and sex	☐	☐
My baby gained weight recently	☐	☐
My baby has always had a high weight	☐	☐
My baby is feeding very vigorously	☐	☐
Family member(s) think my baby is too heavy. If so, who?_____	☐	☐
Other reason. If so, what?_____	☐	☐

_____

YOUR FEEDING ROUTINE

Parents feed their babies in different ways, and we are interested in learning more about how you feed your twins. In the following questions, please think back to your twins' first three months of life

B1. Which of the following best describes each of your twins' eating routine during their first three months?

	I fed my baby whenever he/she cried, got fussy or seemed hungry	My baby was on a flexible feeding schedule (e.g. about every 3-4 hours)	My baby was on a rigid feeding schedule (e.g. I woke him/her up to eat on time)
1st born	☐	☐	☐
2nd born	☐	☐	☐

B2. Did you or the babies decide how often they should feed?

	Me only	Mostly me	Me and my baby equally	Mostly my baby	My baby only
1st born	☐	☐	☐	☐	☐
2nd born	☐	☐	☐	☐	☐

B3. Did you or the babies decide how much milk they should take in a feed?

	Me only	Mostly me	Me and my baby equally	Mostly my baby	My baby only
1st born	☐	☐	☐	☐	☐
2nd born	☐	☐	☐	☐	☐

B4. How would you classify your 'feeding philosophy' for each twin during their first three months?

	Feeding on demand (e.g. fed baby when he/she cried)	Feeding on a schedule (e.g. fed baby at set times)
1st born	☐	☐
2nd born	☐	☐

Now we would like to know more about how your feeding patterns have changed over time

By '**breastfeeding**', we mean any method of feeding breast milk, i.e. feeding directly from breast or giving expressed breast milk in a bottle

By '**bottle-feeding**', we mean feeding formula milk using a bottle

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B5. Which feeding methods did you use in the first three months?

	1st born	2nd born
Entirely breastfeeding	☐	☐
Mostly breastfeeding with some bottle-feeding	☐	☐
Equally breastfeeding and bottle-feeding	☐	☐
Mostly bottle-feeding and some breastfeeding	☐	☐
Almost entirely bottle-feeding (only tried breastfeeding a few times)	☐	☐
Entirely bottle-feeding (never tried breastfeeding)	☐	☐
Other	☐	☐

If other, please describe: _____

_____

If you entirely bottle-fed your twins, please go straight to B10 on page 8

B6. How soon after birth did you start breastfeeding?

1st born	Within.....	_____ minutes	or	_____ hours	or	_____ days
2nd born	Within.....	_____ minutes	or	_____ hours	or	_____ days

B7. How easy was it to establish breastfeeding your twins?

	Very easy	Easy	All right	Difficult	Very difficult
1st born	☐	☐	☐	☐	☐
2nd born	☐	☐	☐	☐	☐

B8. What was your main method of breastfeeding?

	Mostly fed directly from the breast	Equally fed directly from the breast and gave expressed milk	Mostly gave expressed breast milk
1st born	☐	☐	☐
2nd born	☐	☐	☐

B9. Are you currently breastfeeding your twins? Yes, 1st born ☐ Yes, 2nd born ☐ Neither ☐

If you are no longer breastfeeding, when did you stop?

1st born _____ weeks after birth

2nd born _____ weeks after birth

If you entirely breastfed your twins, please go straight to B17 on page 9

B10. How soon after birth did you start bottle-feeding your twins?

1st born	Within.....	_____ minutes	or	_____ hours	or	_____ days
2nd born	Within.....	_____ minutes	or	_____ hours	or	_____ days

B11. Why did you start bottle-feeding?

	1st born	2nd born
Following advice from health professional	☐	☐
Following advice from friends or family	☐	☐
Breastfeeding was too difficult	☐	☐
Baby did not gain enough weight on breast milk alone	☐	☐
Easier to fit into daily routine	☐	☐
Allows other people to feed my baby	☐	☐

If other, please describe _____

_____

B12. Are you currently bottle-feeding your twins?

Yes, 1st born	Yes, 2nd born	Neither
☐	☐	☐

Now we are interested in learning more about <u>how much milk</u> your twins took. Because babies' milk requirements increase as they get older, it is easier to think about how much they took at one specific age. So to answer the questions, please think back to when they were <u>about three months old</u>

If you did not bottle-feed your twins at around 3 months, please go straight to B17 on page 9

B13. What size bottle did you normally use when the twins were <u>about three months old</u>?

	125ml / 4oz	250ml / 9oz	390ml / 12oz	Unsure
1st born	☐	☐	☐	☐
2nd born	☐	☐	☐	☐

B14. How full did you normally fill the bottle?

	Completely full	Mostly full	Half full or less	or	How much formula milk per bottle?
1st born	☐	☐	☐		_____ ml or ____oz
2nd born	☐	☐	☐		_____ ml or ____oz

B15. Most of the time, how much of the bottle did your babies drink?

	All of it	Most of it	Half or less	or	How much formula milk?
1st born	☐	☐	☐		_____ ml or ____oz
2nd born	☐	☐	☐		_____ ml or ____oz

B16. What size teat did you use when the twins were <u>about three months old</u>?

	Fast flow	Medium flow	Slow flow	Variable teat	Unsure
1st born	☐	☐	☐	☐	☐
2nd born	☐	☐	☐	☐	☐

To answer the following questions, please think back to when your twins were <u>about three months old</u>

B17. On average, how many times did you feed your babies during each 24 hour period (one day and one night) when they were <u>about three months old</u>? Please write 0 if you did not breastfeed or bottle-feed your babies when they were about three months old

	Breastfeeding	and / or	Bottle-feeding
1st born	_____ times per day		_____ times per day
2nd born	_____ times per day		_____ times per day

B18. On average, how long did your babies feed for in a typical daytime feed when they were about three months old? Please write 0 if you did not breastfeed or bottle-feed your babies when they were about three months old

	Breastfeeding	and / or	Bottle-feeding
1st born	_____ minutes per feed		_____ minutes per feed
2nd born	_____ minutes per feed		_____ minutes per feed

HOW ACTIVE ARE YOUR TWINS

These questions ask about your twins' physical activity in their first three months of life.
For each behaviour, please indicate how often the baby did this

C1. During feeding, how often did your babies ...		Very rarely	Less than half the time	About half the time	More than half the time	Almost always
lie or sit quietly	1st born	☐	☐	☐	☐	☐
	2nd born	☐	☐	☐	☐	☐
squirm or kick	1st born	☐	☐	☐	☐	☐
	2nd born	☐	☐	☐	☐	☐
wave their arms	1st born	☐	☐	☐	☐	☐
	2nd born	☐	☐	☐	☐	☐

C2. During sleep, how often did your babies ...		Very rarely	Less than half the time	About half the time	More than half the time	Almost always
toss about in the crib	1st born	☐	☐	☐	☐	☐
	2nd born	☐	☐	☐	☐	☐
move from the middle to the end of the crib	1st born	☐	☐	☐	☐	☐
	2nd born	☐	☐	☐	☐	☐
sleep in one position only	1st born	☐	☐	☐	☐	☐
	2nd born	☐	☐	☐	☐	☐

C3. When being dressed or undressed, how often did your babies...		Very rarely	Less than half the time	About half the time	More than half the time	Almost always
wave or kick	1st born	☐	☐	☐	☐	☐
	2nd born	☐	☐	☐	☐	☐
squirm or try to roll away	1st born	☐	☐	☐	☐	☐
	2nd born	☐	☐	☐	☐	☐

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C4. **When put into the bath water, how often did your babies ...**		Very rarely	Less than half the time	About half the time	More than half the time	Almost always
splash or kick	1st born	☐	☐	☐	☐	☐
	2nd born	☐	☐	☐	☐	☐
squirm or turn around	1st born	☐	☐	☐	☐	☐
	2nd born	☐	☐	☐	☐	☐

C5. **When placed on his/her back, how often did your babies ...**		Very rarely	Less than half the time	About half the time	More than half the time	Almost always
wave their arms or kick	1st born	☐	☐	☐	☐	☐
	2nd born	☐	☐	☐	☐	☐
squirm or turn around	1st born	☐	☐	☐	☐	☐
	2nd born	☐	☐	☐	☐	☐

C6. **When placed in a seat (e.g. high chair, push chair, car seat), how often did your babies...**		Very rarely	Less than half the time	About half the time	More than half the time	Almost always
wave their arms or kick	1st born	☐	☐	☐	☐	☐
	2nd born	☐	☐	☐	☐	☐
squirm or turn their body	1st born	☐	☐	☐	☐	☐
	2nd born	☐	☐	☐	☐	☐
sit quietly	1st born	☐	☐	☐	☐	☐
	2nd born	☐	☐	☐	☐	☐

C7. **How old were your babies when they first crawled on hands and knees?**

1st born _____ months Not yet ☐

2nd born _____ months Not yet ☐

C8. **How old were your babies when they could sit up without being supported?**

1st born _____ months Not yet ☐

2nd born _____ months Not yet ☐

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APPETITE

These questions are about your twins' appetite over their first three months of life.
We are specifically interested in the period when your twins were fed milk only,
i.e. no solid foods or pre-prepared baby food yet

D1. How would you rate your twins' appetites in their first three months?

	Poor	OK	Good	Very Good	Excellent
1st born	☐	☐	☐	☐	☐
2nd born	☐	☐	☐	☐	☐

D2. Did either of your twins generally take more milk than the other in their first three months?

1st born took much more milk	1st born took a little more milk	Each took about the same amount	2nd born took a little more milk	2nd born took much more milk
☐	☐	☐	☐	☐

How would you describe your twins' feeding styles at a typical daytime feed in their first three months?

			Never	Rarely	Sometimes	Often	Always
D3.	My baby sucked vigorously	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D4.	My baby sucked steadily and rhythmically	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D5.	My baby seemed contented while feeding	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D6.	My baby frequently wanted more milk than I provided	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D7.	My baby loved milk	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐

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These are some more questions about how your twins feed. Again thinking back to the first three months, please choose which box is most appropriate for each of your babies

			Never	Rarely	Sometimes	Often	Always
D8.	My baby had a big appetite	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D9.	My baby finished feeding quickly	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D10.	My baby became distressed while feeding	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D11.	My baby got full up easily	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D12.	If allowed to, my baby would take too much milk	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D13.	My baby took more than 30 minutes to finish feeding	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D14.	My baby got full before taking all the milk I thought he/she should have had	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D15.	My baby fed slowly	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐

			Never	Rarely	Sometimes	Often	Always
D16.	Even when my baby had just eaten well he/she was happy to feed again if offered	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D17.	My baby found it difficult to manage a complete feed	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D18.	My baby was always demanding a feed	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D19.	My baby sucked more and more slowly during the course of a feed	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D20.	If given the chance, my baby would always be feeding	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D21.	My baby enjoyed feeding time	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
D22.	My baby could easily take a feed within 30 minutes of the last one	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐

HOW YOU FEED YOUR TWINS

The previous section asked some general questions about your feeding.

Now we are interested in learning more about how you fed your twins day-to-day over the first three months. We are particularly interested in whether you changed feeding in different situations

Again, thinking back to the first three months, please choose which box is most appropriate for each of your babies

			Never	Rarely	Sometimes	Often	Always
E1.	I knew when my baby was hungry	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
E2.	I knew when my baby was full	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
E3.	If my baby cried it was usually because he/she was hungry	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
E4.	I worried if my baby did not feed much on one occasion	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
E5.	If my baby wanted to be fed before the next scheduled feed, I fed him/her earlier than usual	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
E6.	When my baby got fussy I tried feeding to settle him/her down	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
E7.	I worried if my baby fed too much on one occasion	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐

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Still thinking back to the first three months, please choose for each of your babies which box is most appropriate

			Never	Rarely	Sometimes	Often	Always
E8.	I gave my baby a large feed to get him/her to sleep longer	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
E9.	I fed my baby to keep him/her quiet when with others	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
E10.	I was careful not to feed my baby too frequently	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
E11.	I was careful not to feed my baby too large an amount	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
If my baby stopped feeding, I ...							
E12.	... tried other methods to encourage him/her e.g. moved baby into a different position or switched breasts	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
E13.	... let him/her have a break then try again a bit later	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
If my baby didn't feed much on one occasion, I ...							
E14.	... made sure he/she took a larger amount at the next feed	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
E15.	... offered him/her another feed a bit sooner than I normally would	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐

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Please only answer these questions if you ever bottle-fed either of your twins during the first three months

If you entirely breastfed your twins, please go straight to F1 on page 18

			Never	Rarely	Sometimes	Often	Always
E16.	I tried to make my baby finish everything in the bottle	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
E17.	If my baby finished the bottle quickly, I made up another	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
E18.	If I worried my baby wasn't eating enough, I added a bit more formula in his/her bottle	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
E19.	If I worried my baby was not feeding enough I changed to a more filling formula	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐

Please only answer these questions if you fed your twins with a mixture of bottle-feeding and breast-feeding during the first three months

If you entirely bottle-fed your twins, please go straight to F1 on page 18

			Never	Rarely	Sometimes	Often	Always
E20.	If my baby was still hungry after a breast-feed, I fed him/her a bottle	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐
E21.	I fed my baby by breast, but gave a bottle before bed to help encourage sleep through the night	1st born	☐	☐	☐	☐	☐
		2nd born	☐	☐	☐	☐	☐

SOLID FOODS

The following section is about solid foods
(i.e. anything other than milk, including mashed up foods and ready prepared baby food)

If neither of your twins has started solid foods, please go straight to F12 on page 21

F1. How old were the twins the very first time solid foods of any kind were eaten (i.e. anything other than milk)?

1st born	____ weeks or ____ months	Not yet ☐
2nd born	____ weeks or ____ months	Not yet ☐

F2. How easy was it to wean your twins onto solid food?

	Very easy	Easy	OK	Difficult	Very difficult
1st born	☐	☐	☐	☐	☐
2nd born	☐	☐	☐	☐	☐

F3. How did you decide to start the twins on solid foods?

	1st born	2nd born
Following advice from health professional	☐	☐
Following advice from friends or family	☐	☐
Milk alone was not enough	☐	☐
Easier to fit into family routine	☐	☐
Baby showed interest in solid foods	☐	☐
Allergy to milk	☐	☐
Other, please describe:	☐	☐

1st born _____

_____

2nd born _____

_____

F4. **In general how much did your baby enjoy starting solid foods?**

	Did not enjoy it at all	Enjoyed it a little	Enjoyed it a lot
1st born	☐	☐	☐
2nd born	☐	☐	☐

F5. **Have your twins started taking solid foods every day?**

	Yes	No	
1st born	☐	☐	If No, please go straight to F8 on this page
2nd born	☐	☐	

F6. **At what age did your twins start taking solid foods every day?**

1st born _____ months old

2nd born _____ months old

F7. **At present, how many times per day does your baby have solid foods?**

1st born _____ times per day

2nd born _____ times per day

F8. **When eating solid food, which of the following statements describes your twins' feeding most accurately?**

	Generally needs to be fully fed by an adult	Generally needs to be fed by an adult but also eats with fingers	Generally eats with spoon but needs help	Generally eats with spoon without help
1st born	☐	☐	☐	☐
2nd born	☐	☐	☐	☐

F9. **When were your twins first given finger foods (i.e. foods babies can pick up and feed to themselves)?**

1st born Age _____ months Not yet ☐

2nd born Age _____ months Not yet ☐

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F10. Has either of your twins tried these foods yet? If so, how old were they when they first tried it?			
		Age when first tried	Not yet tried
Baby rice, cereal, rusks or bread	1st born	_____ months	☐
	2nd born	_____ months	☐
Vegetables (uncooked, cooked or pureed, fresh, frozen or tinned)	1st born	_____ months	☐
	2nd born	_____ months	☐
Chips (e.g. oven fries, smiley faces, potato waffles or wedges)	1st born	_____ months	☐
	2nd born	_____ months	☐
Potatoes or sweet potatoes	1st born	_____ months	☐
	2nd born	_____ months	☐
Processed meat (e.g. sausages, burger)	1st born	_____ months	☐
	2nd born	_____ months	☐
Other meat (e.g. chicken, lamb, pork, beef)	1st born	_____ months	☐
	2nd born	_____ months	☐
Fish (fresh, frozen, tinned or fish fingers)	1st born	_____ months	☐
	2nd born	_____ months	☐
Eggs	1st born	_____ months	☐
	2nd born	_____ months	☐
Dairy products (e.g. milk, cheese, yoghurt)	1st born	_____ months	☐
	2nd born	_____ months	☐
Fizzy drinks with sugar (e.g. 7up, coke)	1st born	_____ months	☐
	2nd born	_____ months	☐
Low calorie fizzy drinks (e.g. 7up zero, diet coke)	1st born	_____ months	☐
	2nd born	_____ months	☐

F11. Has either of your twins tried these foods yet? If so, how old were they when they first tried it?

		Age when first tried	Not yet tried
Squash and/or fruit drinks with sugar (e.g. ribena, robinsons fruit shoot)	1st born	_____ months	☐
	2nd born	_____ months	☐
Low calorie squash and/or fruit drinks (e.g. ribena light, robinsons fruit shoot no added sugar)	1st born	_____ months	☐
	2nd born	_____ months	☐
Pure fruit juice (100% juice)	1st born	_____ months	☐
	2nd born	_____ months	☐
Savoury snacks (e.g. crisps, cheese biscuits)	1st born	_____ months	☐
	2nd born	_____ months	☐
Fruit (uncooked, cooked, pureed, fresh, frozen or tinned)	1st born	_____ months	☐
	2nd born	_____ months	☐
Sweet snacks (e.g. cakes, biscuits, ice cream)	1st born	_____ months	☐
	2nd born	_____ months	☐
Sweets (e.g. chocolate, fruit sweets)	1st born	_____ months	☐
	2nd born	_____ months	☐

F12. Please give the date on which you completed this booklet? ___/___/_____ DD MM YYYY

Thank you

very much for filling in this booklet

PLEASE CHECK that you have given details on YOUR TWINS' GROWTH on page 3 or send us copies of the relevant pages from your twins health records (little red book)

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Space for any additional comments you would like to make

Space for any additional comments you would like to make

Please return both booklets using the freepost envelope and send it to:

Gemini
Health Behaviour Research Centre
Department of Epidemiology & Public Health
UCL
2-16 Torrington Place
London, WC1E 6BT

Appendix 4. Additional tables and figures for Chapter 6 (the Development of the Baby Eating Behaviour Questionnaire)

Appendix 4.1. Questionnaire items included in the first stage of the pilot work

Scale	Item source[a]	BEBQ Item (pilot version)[a]	CEBQ Item[a]
EF	CEBQ	My child loved feeding/mealtimes	My child loves food
	CEBQ	My child was interested in feeding/mealtimes	My child is interested in food
	CEBQ	My child looked forward to feeding/mealtimes	My child looks forward to mealtimes
	CEBQ	My child enjoyed feeding time	My child enjoys eating
	New	My child seemed contented while feeding	NA[b]
	New	My child became distressed while feeding	NA[b]
FR	CEBQ	My child was always crying for a feed	My child is always asking for food
	CEBQ	If allowed to, my child would feed too much	If allowed to, my child would eat too much
	CEBQ	Given the choice, my child would feed most of the time	Given the choice, my child would eat most of the time
	CEBQ	*Even when my child had just eaten well, s/he was happy to feed again if offered*	*Even if my child is full up s/he finds room to eat his/her favourite food*
	CEBQ	If given the chance, my child would always be feeding	If given the chance, my child would always have food in his/her mouth
	New	My child frequently wanted more milk than I could provide	NA[b]
SR	CEBQ	My child had a big appetite	My child has a big appetite
	CEBQ	*My child didn't seem to drink all of the milk I was able to provide*	*My child leaves food on his/her plate at the end of a meal*
	CEBQ	My child got full before I finished feeding him/her	My child gets full before his/her meal is finished
	CEBQ	My child got full up easily	My child gets full up easily
	CEBQ	My child could not take a feed if s/he had had one shortly before	My child cannot eat a meal if s/he has had a snack just before
SE	CEBQ	My child finished feeding quickly	My child finishes his/her meal quickly
	CEBQ	My child fed slowly	My child eats slowly
	CEBQ	My child took more than 30 minutes to finish feeding	My child takes more than 30 minutes to finish a meal
	CEBQ	My child drank more and more slowly during the course of a feed	My child eats more and more slowly during the course of a meal
EOE	CEBQ	My child fed more when s/he was irritable	My child eats more when worried
	CEBQ	My child fed more when s/he was grumpy	My child eats more when annoyed
	CEBQ	My child fed more when s/he was anxious	My child eats more when anxious
	CEBQ	My child fed more when s/he had nothing else to do	My child eats more when s/he has nothing else to do
EUE	CEBQ	My child fed less when s/he was grumpy	My child eats less when angry
	CEBQ	My child fed less when s/he was tired	My child eats less when s/he is tired
	CEBQ	My child fed more when s/he was happy	My child eats more when she is happy
	CEBQ	My child fed less when s/he was upset	My child eats less when upset
DD	CEBQ	My child was always crying for a drink	My child is always asking for a drink
	CEBQ	If given the chance my child would drink continuously throughout the day	If given the chance, my child would drink continuously throughout the day
	CEBQ	If given the chance my child would always be having a drink	If given the chance, my child would always be having a drink

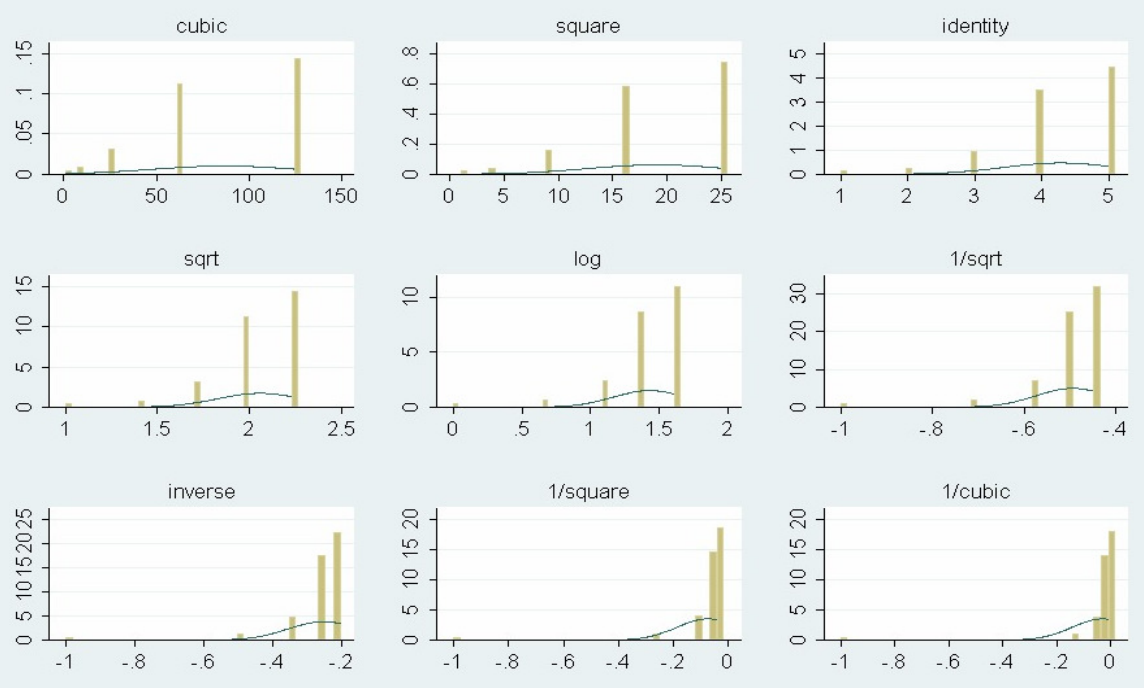
Abbreviations: BEBQ, Baby Eating Behaviour Questionnaire; CEBQ, Child Eating Behaviour Questionnaire; EF, 'enjoyment of food'; FR, 'food responsiveness'; SR, 'satiety responsiveness'; SE, 'slowness in eating'; EOE, 'emotional overeating'; EUE, 'emotional under-eating'; DD, 'desire to drink'.
[a]Items that were modified substantially for the pilot work are italicized, and items not based on CEBQ items, but created for the milk-feeding period are labelled 'new'.
[b]NA, not applicable.

Appendix 4.2. Scales and items included in the questionnaire for the quantitative pilot work and original CEBQ scales on which they were based

BEBQ Scale	Source	Items in the Pilot Questionnaire
EF	CEBQ	My baby loved milk
	CEBQ	My baby enjoyed feeding time
	New	My baby seemed contented while feeding
	New	My baby became distressed while feeding
FR	CEBQ	My baby was always demanding a feed
	CEBQ	If allowed to, my baby would take too much milk
	CEBQ	Even when my baby had just eaten well, s/he was happy to feed again if offered
	CEBQ	If given the chance, my baby would always be feeding
	CEBQ	Given the choice my baby would feed most of the time[a]
	New	My baby frequently wanted more milk than I provided
SR	CEBQ	My baby found it difficult to manage a complete feed
	CEBQ	My baby got full before taking all the milk I thought s/he should have
	CEBQ	My baby got full up easily
	CEBQ	My baby could easily take a feed within 30 minutes of the last one
	CEBQ	My baby had a big appetite
SE	CEBQ	My baby finished feeding quickly
	CEBQ	My baby fed slowly
	CEBQ	My baby took more than 30 minutes to finish feeding
	CEBQ	My baby sucked more and more slowly during the course of a feed

Abbreviations: BEBQ, Baby Eating Behaviour Questionnaire; CEBQ, Child Eating Behaviour Questionnaire; EF, 'enjoyment of food'; FR, 'food responsiveness'; SR, 'satiety responsiveness'; SE, 'slowness in eating'.
[a] This item was removed from the pool following the quantitative pilot work.

Appendix 4.3. Unsuccessful transformations of 'my baby seems contented while feeding'



Appendix 4.4. Skewness and kurtosis statistics for the Baby Eating Behaviour Questionnaire subscales, and 'appetite size'

BEBQ Scale	Skewness (SE)	Kurtosis (SE)
'Enjoyment of Food'	-1.43 (0.05)	2.58 (0.10)
'Food Responsiveness'	0.93 (0.05)	0.97 (0.10)
'Slowness in Eating'	0.29 (0.05)	-0.31 (0.10)
'Satiety Responsiveness'	0.47 (0.05)	0.01 (0.10)
'Appetite Size'	-0.12 (0.05)	-0.55 (0.10)

Appendix 5. Additional tables and figures for Chapter 8 (Genetic and environmental influences on appetitive traits in infancy)

Appendix 5.1. Within-pair intraclass correlations (95% confidence intervals) for Baby Eating Behaviour Questionnaire subscale scores: all twins, with additional adjustment for gestational-age, without 'premature' infants, and without 'problem-feeders'

BEBQ Scale[1]	Intraclass Correlations (95% Confidence Intervals) for BEBQ scale scores							
	All twin pairs[2]		With adjustment for gestational age[3]		Excluding infants born < 34 weeks[4]		Excluding 'problem-feeders'[4]	
	MZs	DZs	MZs	DZs	MZs	DZs	MZs	DZs
EF	**0.82** (0.80-0.85)	**0.41** (0.37-0.45)	**0.82 (**0.80-0.84)	**0.41** (0.36-0.45)	**0.82** (0.80-0.85)	**0.38** (0.33-0.42)	**0.86** (0.83-0.89)	**0.49** (0.44-0.54)
N[5]	712	1576	710	1569	586	1389	372	875
FR	**0.88** (0.86-0.89)	**0.60** (0.56-0.63)	**0.88** (0.86-0.89)	**0.59** (0.55-0.62)	**0.89** (0.88-0.91)	**0.59** (0.55-0.62)	**0.91** (0.89-0.93)	**0.62** (0.58-0.66)
N[5]	716	1576	714	1569	591	1390	375	879
SR	**0.83** (0.81-0.85)	**0.49** (0.45-0.53)	**0.83** (0.81-0.85)	**0.48** (0.44-0.52)	**0.84** (0.82-0.86)	**0.48** (0.44-0.52)	**0.88** (0.85-0.90)	**0.53** (0.48-0.57)
N[5]	718	1582	716	1575	592	1393	377	883
SE	**0.84** (0.82-0.86)	**0.41** (0.37-0.45)	**0.84** (0.82-0.86)	**0.40** (0.36-0.44)	**0.84** (0.81-0.86)	**0.39** (0.35-0.44)	**0.88** (0.85-0.90)	**0.40** (0.34-0.45)
N[5]	719	1583	717	1576	593	1395	376	880
AS	**0.76** (0.73-0.79)	**0.40** (0.36-0.44)	**0.76** (0.73-0.79)	**0.38** (0.33-0.42)	**0.75** (0.72-0.79)	**0.38** (0.33-0.42)	**0.81** (0.77-0.84)	**0.43** (0.37-0.48)
N[5]	718	1587	716	1580	592	1398	376	883

[1] Abbreviations: BEBQ, Baby Eating Behaviour Questionnaire; EF, 'enjoyment of food'; FR, 'food responsiveness'; 'SR, satiety responsiveness'; SE, 'slowness in eating'; AS, 'appetite size'.
[2] Within-pair correlations using BEBQ scale scores regressed on sex and age in months when the BEBQ was completed by the parents.
[3] Within-pair correlations using BEBQ scale scores regressed on sex, age in months when the BEBQ was completed by the parents, and gestational age in weeks.
[4] Within-pair correlations using BEBQ scale scores regressed on sex and age in months when the BEBQ was completed by the parents.
[5] Number of twin pairs.

Appendix 5.2. Sensitivity analyses for 'enjoyment of food'

Data (*n*)	Model	Additive Genetic Effect (a^2)	Shared Environment Effect (c^2)	Non-shared Environment Effect (e^2)	-2LL	df	Reference Model	$\Delta\chi^2$ (df)	*P*	Δ AIC	Δ BIC
1	**Sat**	-	-	-	11851.795	4571	-	-	-	-	
(4581)	**ACE**	0.83 (0.76-0.85)	0.00 (0.00-0.06)	0.17 (0.15-0.19)	11877.612	4576	Sat	25.817 (5)	<0.001	15.817	-6.436
	CE	-	0.53 (0.50-0.56)	0.47 (0.44-0.50)	12205.134	4577	ACE	327.522 (1)	<0.001	325.522	159.892
	AE	**0.83 (0.81-0.85)**	**-**	**0.17 (0.15-0.19)**	**11877.612**	**4577**	**ACE**	**<0.001 (1)**	**1**	**-2**	**-3.869**
	E	-	-	1.00 (1.00-1.00)	12972.814	4578	ACE	1095.201 (2)	<0.001	1091.201	539.863
2	**Sat**	-	-	-	11807.105	4553	-	-	-	-	
(4563)	**ACE**	0.83 (0.77-0.85)	0.00 (0.00-0.06)	0.17 (0.15-0.19)	11837.776	45558	Sat	30.671 (5)	<0.001	20.671	-3.999
	CE	-	0.53 (0.50-0.56)	0.47 (0.44-0.50)	12165.175	4559	ACE	327.398 (1)	<0.001	325.398	159.833
	AE	**0.83 (0.81-0.85)**	**-**	**0.17 (0.15-0.19)**	**11837.776**	**4559**	**ACE**	**<0.001 (1)**	**1**	**-2**	**-3.867**
	E	-	-	1.00 (1.00-1.00)	12918.263	4560	ACE	1080.487 (2)	<0.001	1076.487	532.51
3	**Sat**	-	-	-	10177.559	3945	-	-	-	-	
(3955)	**ACE**	0.83 (0.79-0.85)	0.00 (0.00-0.03)	0.17 (0.15-0.19)	10222.686	3950	Sat	45.127 (5)	<0.001	35.127	-145.765
	CE	-	0.53 (0.47-0.54)	0.50 (0.46-0.53)	10521.806	3951	ACE	299.120 (1)	<0.001	297.12	145.765
	AE	**0.83 (0.81-0.85)**	**-**	**0.17 (0.15-0.19)**	**10222.686**	**3951**	**ACE**	**<0.001 (1)**	**1**	**-2**	**-3.795**
	E	-	-	1.00 (1.00-1.00)	11100.525	3952	ACE	877.839 (2)	<0.001	873.839	431.329
4	**Sat**	-	-	-	6443.827	2814	-	-	-	-	
(2824)	**ACE**	0.81 (0.71-0.89)	0.08 (0.00-0.17)	0.12 (0.10-0.14)	6464.734	2819	Sat	20.907 (5)	0.001	10.907	-7.955
	CE	-	0.59 (0.55-0.62)	0.41 (0.38-0.45)	6692.846	2820	ACE	228.111 (1)	<0.001	226.111	110.375
	AE	**0.88 (0.87-0.90)**	**-**	**0.12 (0.10-0.13)**	**6467.167**	**2820**	**ACE**	**2.433 (1)**	**0.119**	**0.433**	**-2.465**
	E	-	-	1.00 (1.00-1.00)	7225.081	2821	ACE	760.347 (2)	<0.001	756.347	372.811

Abbreviations: -2LL, -2 log likelihood; df, degrees of freedom; Reference Model, the model that the goodness of fit statistics are compared to; $\Delta\chi^2$(df), change in Chi-square statistic (degrees of freedom); *P*, significance value; ΔAIC, change in Aikaike's Information Criterion statistic; ΔBIC, change in Bayesian Information Criterion statistic.
Data: 1, includes all twins and scores are adjusted for age at BEBQ completion and sex; 2, includes all twins and scores are adjusted for age at BEBQ completion, sex and gestational age; 3, excludes twin pairs born before 34 weeks gestation, and scores are adjusted for age at BEBQ completion and sex; 4, excludes infants with any reported feeding problem, and scores are adjusted for age at BEBQ completion and sex.
Model: CE, AE and E models are nested within the full ACE model. The ACE model dissects the phenotypic variance into a^2, c^2 and e^2; the CE model drops the a^2 parameter and assesses the variance explained by the c^2 and e^2 parameters only; the AE model drops the c^2 parameter and assesses the variance explained by the a^2 and e^2 parameters only; the E model drops both the a^2 and c^2 parameters and assesses the variance explained by e^2 only. The most parsimonious model for each analysis is bolded.

Appendix 5.3. Sensitivity analyses for 'food responsiveness'

Data (*n*)	Model	Additive Genetic Effect (a^2)	Shared Environment Effect (c^2)	Non-shared Environment Effect (e^2)	-2LL	df	Reference Model	$\Delta\chi^2$ (df)	*P*	Δ AIC	Δ BIC
1	**Sat**	-	-	-	11237.636	4577	-	-	-	-	
(4587)	**ACE**	**0.59 (0.52-0.65)**	**0.30 (0.24-0.36)**	**0.11 (0.10-0.13)**	**11242.871**	**4582**	**Sat**	**5.235 (5)**	**0.388**	**-4.765**	**-16.729**
	CE	-	0.68 (0.66-0.70)	0.32 (0.30-0.34)	11567.955	4583	ACE	325.084(1)	<0.001	323.084	158.673
	AE	0.89 (0.87-0.90)	-	0.11 (0.10-0.13)	11312.857	4583	ACE	69.986(1)	<0.001	67.986	31.124
	E	-	-	1.00 (1.00-1.00)	12987.508	4584	ACE	1744.637(2)	<0.001	1740.637	864.58
2	**Sat**	-	-	-	11218.883	4559	-	-	-	-	
(4569)	**ACE**	**0.60 (0.53-0.66)**	**0.28 (0.22-0.34)**	**0.12 (0.11-0.13)**	**11222.798**	**4564**	**Sat**	**3.915 (5)**	**0.562**	**-6.085**	**-17.378**
	CE	-	0.67 (0.65-0.70)	0.32 (0.30-0.35)	11548.422	4565	ACE	325.624(1)	<0.001	323.624	158.945
	AE	0.88 (0.87-0.89)	-	0.12 (0.10-0.13)	11285.385	4565	ACE	62.587(1)	<0.001	60.587	27.426
	E	-	-	1.00 (1.00-1.00)	12932.074	4566	ACE	1709.276	<0.001	1705.276	846.903
3	**Sat**	-	-	-	9697.559	3955	-	-	-	-	
(3965)	**ACE**	**0.63 (0.56-0.70)**	**0.27 (0.20-0.34)**	**0.10 (0.09-0.14)**	**9701.335**	**3960**	**Sat**	**3.776 (5)**	**0.582**	**-6.224**	**-17.095**
	CE	-	0.68 (0.65-0.70)	0.32 (0.30-0.35)	10032.276	3961	ACE	330.941 (1)	<0.001	328.941	161.674
	AE	0.90 (0.89-0.91)	-	0.10 (0.09-0.11)	9753.026	3961	ACE	51.691 (1)	<0.001	49.691	22.049
	E	-	-	1.00 (1.00-1.00)	11242.534	3962	ACE	1541.199 (1)	<0.001	1537.199	763.007
4	**Sat**	-	-	-	6973.120	2825	-	-	-	-	
(2835)	**ACE**	**0.59 (0.52-0.67)**	**0.32 (0.25-0.40)**	**0.09 (0.07-0.10)**	**6978.465**	**2830**	**Sat**	**5.345 (5)**	**0.375**	**-4.655**	**-15.742**
	CE	-	0.79 (0.68-0.73)	0.30-0.32)	7207.691	2831	ACE	229.226(1)	<0.001	227.226	110.93
	AE	0.91 (0.90-0.93)	-	0.09 (0.07-0.10)	7028.982	2831	ACE	50.517(1)	<0.001	48.517	21.576
	E	-	-	1.00 (1.00-1.00)	8068.668	2832	ACE	1090.203(2)	<0.001	1086.203	537.736

Abbreviations: -2LL, -2 log likelihood; df, degrees of freedom; Reference Model, the model that the goodness of fit statistics are compared to; $\Delta\chi^2$(df), change in Chi-square statistic (degrees of freedom); *P*, significance value; ΔAIC, change in Aikaike's Information Criterion statistic; ΔBIC, change in Bayesian Information Criterion statistic.

Data: 1, includes all twins and scores are adjusted for age at BEBQ completion and sex; 2, includes all twins and scores are adjusted for age at BEBQ completion, sex and gestational age; 3, excludes twin pairs born before 34 weeks gestation, and scores are adjusted for age at BEBQ completion and sex; 4, excludes infants with any reported feeding problem, and scores are adjusted for age at BEBQ completion and sex.

Model: CE, AE and E models are nested within the full ACE model. The ACE model dissects the phenotypic variance into a^2, c^2 and e^2; the CE model drops the a^2 parameter and assesses the variance explained by the c^2 and e^2 parameters only; the AE model drops the c^2 parameter and assesses the variance explained by the a^2 and e^2 parameters only; the E model drops both the a^2 and c^2 parameters and assesses the variance explained by e^2 only. The most parsimonious model for each analysis is bolded.

Appendix 5.4. Sensitivity analyses for 'slowness in eating'

Data (*n*)	Model	Additive Genetic Effect (a^2)	Shared Environment Effect (c^2)	Non-shared Environment Effect (e^2)	-2LL	df	Reference Model	$\Delta\chi^2$ (df)	*P*	Δ AIC	Δ BIC
1	**Sat**	-	-	-	11880.516	4599	-	-	-	-	
(4609)	**ACE**	0.84 (0.79-0.86)	0.00 (0.00-0.05)	0.16 (0.14-0.17)	11885.701	4604	Sat	5.185 (5)	0.394	-4.815	-16.766
	CE	-	0.54 (0.51-0.57)	0.46 (0.43-0.49)	12265.973	4605	ACE	380.272(1)	<0.001	378.272	186.264
	AE	**0.84 (0.83-0.86)**	**-**	**0.16 (0.14-0.17)**	**11885.701**	**4605**	**ACE**	**0.000(1)**	**-***	**-2.000**	**-3.872**
	E	-	-	1.00 (1.00-1.00)	13063.004	4606	ACE	1177.303(2)	<0.001	1173.303	580.907
2	**Sat**	-	-	-	11871.296	4581	-	-	-	-	
(4591)	**ACE**	0.84 (0.79-0.86)	0.00 (0.00-0.04)	0.16 (0.14-0.18)	11873.836	4586	Sat	2.540 (5)	0.771	-7.460	-18.08
	CE	-	0.53 (0.50-0.56)	0.45 (0.44-0.49)	12250.580	4587	ACE	376.745(1)	<0.001	374.745	184.503
	AE	**0.84 (0.82-0.86)**	**-**	**0.16 (0.14-0.18)**	**11873.836**	**4587**	**ACE**	**0.000**	**-***	**-2.000**	**-3.87**
	E	-	-	1.00 (1.00-1.00)	13023.304	4588	ACE	1149.469(2)	<0.001	1145.469	566.995
3	**Sat**	-	-	-	10122.136	3971	-	-	-	-	
(3981)	**ACE**	0.84 (0.80-0.86)	0.00 (0.00-0.04)	0.16 (0.14-0.18)	10131.009	3976	Sat	8.873 (5)	0.114	-1.127	-14.556
	CE	-	0.52 (0.49-0.55)	0.48 (0.45-0.51)	10456.412	3977	ACE	325.403 (1)	<0.001	323.403	158.902
	AE	**0.84 (0.82-0.86)**	**-**	**0.16 (0.14-0.18)**	**10131.009**	**3977**	**ACE**	**0.000 (1)**	**-***	**-2.000**	**-3.799**
	E	-	-	1.00 (1.00-1.00)	11086.681	3978	ACE	955.672 (2)	<0.001	951.672	470.238
4	**Sat**	-	-	-	7020.160	2837	-	-	-	-	
(2847)	**ACE**	0.88 (0.83-0.90)	0.00 (0.00-0.05)	0.12 (0.10-0.14)	7030.394	2842	Sat	10.234 (5)	0.069	0.234	-13.307
	CE	-	0.54 (0.50-0.58)	0.46 (0.42-0.50)	7301.977	2843	ACE	271.584(1)	<0.001	269.584	132.107
	AE	**0.88 (0.86-0.90)**	**-**	**0.12 (0.10-0.14)**	**7030.394**	**2843**	**ACE**	**0.000(1)**	**-****	**-2.000**	**-3.684**
	E	-	-	1.00 (1.00-1.00)	7731.305	2844	ACE	700.911(2)	<0.001	696.911	343.086

Abbreviations: -2LL, -2 log likelihood; df, degrees of freedom; Reference Model, the model that the goodness of fit statistics are compared to; $\Delta\chi^2$(df), change in Chi-square statistic (degrees of freedom); *P*, significance value; ΔAIC, change in Aikaike's Information Criterion statistic; ΔBIC, change in Bayesian Information Criterion statistic.

Data: 1, includes all twins and scores are adjusted for age at BEBQ completion and sex; 2, includes all twins and scores are adjusted for age at BEBQ completion, sex and gestational age; 3, excludes twin pairs born before 34 weeks gestation, and scores are adjusted for age at BEBQ completion and sex; 4, excludes infants with any reported feeding problem, and scores are adjusted for age at BEBQ completion and sex.

Model: CE, AE and E models are nested within the full ACE model. The ACE model dissects the phenotypic variance into a^2, c^2 and e^2; the CE model drops the a^2 parameter and assesses the variance explained by the c^2 and e^2 parameters only; the AE model drops the c^2 parameter and assesses the variance explained by the a^2 and e^2 parameters only; the E model drops both the a^2 and c^2 parameters and assesses the variance explained by e^2 only. The most parsimonious model for each analysis is bolded.

Appendix 5.5. Sensitivity analyses for 'satiety responsiveness'

Data (*n*)	Model	Additive Genetic Effect (a^2)	Shared Environment Effect (c^2)	Non-shared Environment Effect (e^2)	-2LL	df	Reference Model	$\Delta\chi^2$ (df)	*P*	Δ AIC	Δ BIC
1	**Sat**	-	-	-	11735.078	4593	-	-	-	-	
(4603)	**ACE**	**0.72 (0.65-0.80)**	**0.12 (0.05-0.19)**	**0.16 (0.14-0.17)**	**11740.627**	**4598**	**Sat**	**5.549 (5)**	**0.353**	**-4.451**	**-16.581**
	CE	-	0.59 (0.56-0.62)	0.41 (0.38-0.44)	12043.542	4599	ACE	302.915(1)	<0.001	300.915	147.587
	AE	0.85 (0.83-0.86)	-	0.15 (0.14-0.17)	22750.796	4599	ACE	10.169(1)	0.001	8.169	1.214
	E	-	-	1.00 (1.00-1.00)	13025.624	4600	ACE	1284.997(2)	<0.001	1280.997	634.757
2	**Sat**	-	-	-	11713.831	4575	-	-	-	-	
(4585)	**ACE**	**0.73 (0.66-0.81)**	**0.11 (0.03-0.18)**	**0.16 (0.14-0.18)**	**11718.306**	**4580**	**Sat**	**4.475 (5)**	**0.483**	**-5.525**	**-17.108**
	CE	-	0.58 (0.56-0.61)	0.42 (0.39-0.44)	12021.379	4581	ACE	303.074(1)	<0.001	301.074	147.668
	AE	0.84 (0.83-0.86)	-	0.16 (0.14-0.17)	11726.084	4581	ACE	7.779(1)	0.005	5.779	0.02
	E	-	-	1.00 (1.00-1.00)	12973.720	4582	ACE	1255.415(2)	<0.001	1251.415	619.969
3	**Sat**	-	-	-	10076.650	3963	-	-	-	-	
(3973)	**ACE**	**0.76 (0.68-0.84)**	**0.09 (0.01-0.17)**	**0.14 (0.13-0.16)**	**10084.957**	**3968**	**Sat**	**8.307 (5)**	**0.140**	**-1.693**	**-14.834**
	CE	-	0.58 (0.55-0.61)	0.42 (0.39-0.45)	10370.849	3969	ACE	285.892 (1)	<0.001	283.892	139.148
	AE	0.86 (0.84-0.87)	-	0.14 (0.13-0.16)	10090.273	3969	ACE	5.316 (1)	<0.001	3.316	-1.14
	E	-	-	1.00 (1.00-1.00)	11188.091	3970	ACE	1103.135 (2)	<0.001	1099.135	543.972
4	**Sat**	-	-	-	6873.499	2829	-	-	-	-	
(2839)	**ACE**	**0.74 (0.65-0.83)**	**0.15 (0.06-0.24)**	**0.11 (0.10-0.13)**	**6883.523**	**2834**	**Sat**	**10.024 (5)**	**0.075**	**0.024**	**-13.406**
	CE	-	0.62 (0.59-0.65)	0.38 (0.35-0.41)	7104.057	2835	ACE	220.534(1)	<0.001	218.534	106.584
	AE	0.89 (0.87-0.90)	-	0.11 (0.10-0.13)	6892.916	2835	ACE	9.394(1)	0.002	7.394	1.013
	E	-	-	1.00 (1.00-1.00)	7719.220	2836	ACE	835.698(2)	<0.001	831.698	410.482

Abbreviations: -2LL, -2 log likelihood; df, degrees of freedom; Reference Model, the model that the goodness of fit statistics are compared to; $\Delta\chi^2$(df), change in Chi-square statistic (degrees of freedom); *P*, significance value; ΔAIC, change in Aikaike's Information Criterion statistic; ΔBIC, change in Bayesian Information Criterion statistic.

Data: 1, includes all twins and scores are adjusted for age at BEBQ completion and sex; 2, includes all twins and scores are adjusted for age at BEBQ completion, sex and gestational age; 3, excludes twin pairs born before 34 weeks gestation, and scores are adjusted for age at BEBQ completion and sex; 4, excludes infants with any reported feeding problem, and scores are adjusted for age at BEBQ completion and sex.

Model: CE, AE and E models are nested within the full ACE model. The ACE model dissects the phenotypic variance into a^2, c^2 and e^2; the CE model drops the a^2 parameter and assesses the variance explained by the c^2 and e^2 parameters only; the AE model drops the c^2 parameter and assesses the variance explained by the a^2 and e^2 parameters only; the E model drops both the a^2 and c^2 parameters and assesses the variance explained by e^2 only. The most parsimonious model for each analysis is bolded.

Appendix 5.6. Sensitivity analyses for 'appetite size'

Data (*n*)	Model	Additive Genetic Effect (a^2)	Shared Environment Effect (c^2)	Non-shared Environment Effect (e^2)	-2LL	df	Reference Model	$\Delta\chi^2$ (df)	*P*	Δ AIC	Δ BIC
1	**Sat**	-	-	-	12165.658	4604	-	-	-	-	-
(4614)	**ACE**	0.73 (0.64-0.79)	0.03 (0.00-0.11)	0.23 (0.21-0.26)	12170.948	4609	Sat	5.290 (5)	0.382	-4.710	-16.716
	CE	-	0.51 (0.48-0.54)	0.49 (0.46-0.52)	12374.457	4610	ACE	203.509 (1)	<0.001	201.509	97.882
	AE	**0.77 (0.74-0.79)**	**-**	**0.23 (0.21-0.26)**	**12171.475**	**4610**	**ACE**	**0.528 (1)**	**0.468**	**-1.472**	**-3.609**
	E	-	-	1.00 (1.00-1.00)	13073.423	4611	ACE	902.475 (2)	<0.001	898.475	443.493
2	**Sat**	-	-	-	12164.905	4586	-	-	-	-	-
(4596)	**ACE**	0.76 (0.67-0.78)	000 (0.00-0.08)	0.24 (022-0.27)	12166.004	4591	Sat	1.099 (5)	0.954	-8.901	-18.802
	CE	-	0.50 (0.47-0.53)	0.50 (0.47-0.53)	12375.719	4592	ACE	209.714 (1)	<0.001	207.714	100.987
	AE	**0.76 (0.73-0.78)**	**-**	**0.24 (0.22-0.27)**	**12166.004**	**4592**	**ACE**	**0.000 (1)**	*	**-2.000**	**-3.870**
	E	-	-	1.00 (1.00-1.00)	13029.665	4593	ACE	863.661 (2)	<0.001	859.661	424.09
3	**Sat**	-	-	-	10459.299	3974	-	-	-	-	-
(3984)	**ACE**	0.75 (0.65-0.78)	0.00 (0.00-0.09)	0.25 (0.22-0.28)	10465.444	3979	Sat	6.145 (5)	0.292	-3.855	-15.922
	CE	-	0.49 (0.46-0.52)	0.51 (0.48-0.54)	10631.461	3980	ACE	166.017 (1)	<0.001	164.017	79.21
	AE	**0.75 (0.72-0.78)**	**-**	**0.25 (0.22-0.28)**	**10465.454**	**3980**	**ACE**	**0.011 (1)**	**0.918**	**-1.989**	**-3.794**
	E	-	-	1.00 (1.00-1.00)	11179.381	3981	ACE	713.937 (2)	<0.001	709.937	349.371
4	**Sat**	-	-	-	7336.702	2836	-	-	-	-	-
(2846)	**ACE**	0.75 (0.64-0.83)	0.06 (0.00-0.16)	0.19 (0.17-0.23)	7341.552	2841	Sat	4.850 (5)	0.434	-5.150	-15.999
	CE	-	0.54 (0.50-0.58)	0.46 (0.42-0.50)	7476.619	2842	ACE	135.066 (1)	<0.001	133.066	63.849
	AE	**0.81 (0.78-0.83)**	**-**	**0.19 (0.17-0.22)**	**7342.616**	**2842**	**ACE**	**1.064 (1)**	**0.302**	**-0.936**	**-3.153**
	E	-	-	1.00 (1.00-1.00)	7915.602	2843	ACE	574.050 (2)	<0.001	570.050	279.656

Abbreviations: -2LL, -2 log likelihood; df, degrees of freedom; Reference Model, the model that the goodness of fit statistics are compared to; $\Delta\chi^2$(df), change in Chi-square statistic (degrees of freedom); *P*, significance value; ΔAIC, change in Aikaike's Information Criterion statistic; ΔBIC, change in Bayesian Information Criterion statistic.

Data: 1, includes all twins and scores are adjusted for age at BEBQ completion and sex; 2, includes all twins and scores are adjusted for age at BEBQ completion, sex and gestational age; 3, excludes twin pairs born before 34 weeks gestation, and scores are adjusted for age at BEBQ completion and sex; 4, excludes infants with any reported feeding problem, and scores are adjusted for age at BEBQ completion and sex.

Model: CE, AE and E models are nested within the full ACE model. The ACE model dissects the phenotypic variance into a2, c2 and e2; the CE model drops the a2 parameter and assesses the variance explained by the c2 and e2 parameters only; the AE model drops the c2 parameter and assesses the variance explained by the a2 and e2 parameters only; the E model drops both the a2 and c2 parameters and assesses the variance explained by e2 only. The most parsimonious model for each analysis is bolded.

Appendix 5.7. Within-pair intraclass correlations (95% confidence intervals) for Baby Eating Behaviour Questionnaire subscale scores for 'correctly' and 'incorrectly' classified twins

Baby Eating Behaviour Questionnaire Scale	MZs[1]		DZs[1]	
	MZQ-MZP	MZQ-DZP	DZQ-DZP	DZQ-MZP
'Enjoyment of food'	**0.81** (0.78-0.86)	**0.86** (0.81-0.88)	**0.41** (0.36-0.45)	**0.62** (0.20-0.85)
n	504	208	1560	16
'Food responsiveness'	**0.90** (0.88-0.92)	**0.82** (0.77-0.86)	**0.59** (0.56-0.63)	**0.65** (0.26-0.86)
n	505	211	1560	16
'Slowness in eating'	**0.84** (0.81-0.86)	**0.81** (0.76-0.85)	**0.41** (0.37-0.45)	**0.27** (-0.24-0.66)
n	507	211	1567	16
'Satiety responsiveness'	**0.84** (0.81-0.86)	**0.84** (0.80-0.88)	**0.49** (0.45-0.53)	**0.64** (0.24-0.86)
n	507	212	1566	16
'Appetite size'	**0.78** (0.73-0.81)	**0.73** (0.66-0.79)	**0.40** (0.35-0.44)	**0.47** (-0.02-0.78)
n	505	213	1571	16

Abbreviations: MZs, all pairs classified as MZ by the zygosity questionnaire; DZs, all pairs classified as DZ by the zygosity questionnaire; MZQ-MZP, pairs classified as MZ by both the zygosity questionnaire and the parents; MZQ-DZPs, pairs classified as MZ by the zygosity questionnaire and as DZ by the parents; DZQ-DZP, pairs classified as DZ by both the zygosity questionnaire and the parents; DZQ-MZP, pairs classified as DZ by the zygosity questionnaire and as MZ by the parents.

[1] Within-pair correlations using Baby Eating Behaviour Questionnaire (BEBQ) scale scores regressed on sex and age in months when the BEBQ was completed by the parents.

Appendix 5.8. Parameter estimates for calculating heritability with 'correctly' and 'incorrectly' classified MZs, and 'correctly' classified DZs

BEBQ scale[1]	DZs & MZQ-MZPs			DZs & MZQ-DZPs			MZs and DZQ-DZPs		
	A	C	E	A	C	E	A	C	E
EF	**0.81** (0.72-0.83)	**0.00** (0.00-0.08)	**0.19** (0.17-0.21)	**0.87** (0.82-0.89)	**0.00** (0.00-0.04)	**0.13** (0.11-0.17)	**0.81** (0.72-0.83)	**0.00** (0.00-0.08)	**0.19** (0.17-0.21)
FR	**0.61** (0.55-0.68)	**0.29** (0.22-0.35)	**0.10** (0.09-0.11)	**0.50** (0.41-0.58)	**0.34** (0.27-0.41)	**0.16** (0.13-0.19)	**0.61** (0.55-0.68)	**0.29** (0.22-0.35)	**0.10** (0.09-0.11)
SE	**0.85** (0.79-0.86)	**0.00** (0.00-0.05)	**0.15** (0.14-0.18)	**0.84** (0.76-0.87)	**0.00** (0.00-0.06)	**0.16** (0.13-0.20)	**0.85** (0.79-0.86)	**0.00** (0.00-0.05)	**0.15** (0.14-0.18)
SR	**0.72** (0.64-0.80)	**0.13** (0.05-0.20)	**0.15** (0.14-0.18)	**0.73** (0.64-0.82)	**0.12** (0.04-0.19)	**0.15** (0.12-0.18)	**0.72** (0.64-0.80)	**0.13** (0.05-0.20)	**0.15** (0.14-0.18)
AS	**0.75** (0.65-0.80)	**0.03** (0.00-0.11)	**0.23** (0.20-0.26)	**0.70** (0.57-0.79)	**0.04** (0.00-0.13)	**0.25** (0.21-0.31)	**0.75** (0.65-0.80)	**0.03** (0.00-0.11)	**0.23** (0.20-0.26)

Abbreviations:BEBQ, Baby Eating Behaviour Questionnaire; EF, 'enjoyment of food'; FR, 'food responsiveness'; SE, 'slowness in eating'; SR, 'satiety responsiveness'; AS, 'appetite size'; DZs, all pairs classified as DZ by the zygosity questionnaire; MZs, all pairs classified as MZ by the zygosity questionnaire; MZQ-MZPs, pairs classified as MZ by both the zygosity questionnaire and the parents; MZQ-DZPs, pairs classified as MZ by the zygosity questionnaire and as DZ by the parents; DZQ-DZP, pairs classified as DZ by both the zygosity questionnaire and the parents; A, additive genetic effect; C, shared environment effect; E, unique environment effect.

[1] BEBQ scale scores were regressed on sex and age in months when the BEBQ was completed by the parents.

Appendix 5.9. Within-pair tetrachoric correlations (95% confidence intervals) for 'enjoyment of food' as a dichotomous variable: all twins, with additional adjustment for gestational-age, without 'premature' infants, and without 'problem-feeders'

Tetrachoric Correlations (95% Confidence Intervals) N[3]							
All twin pairs[1]		With adjustment for gestational age[2]		Excluding infants born < 34 weeks[1]		Excluding 'problem-feeders'[1]	
MZs	DZs	MZs	DZs	MZs	DZs	MZs	DZs
0.97 (0.96-0.99)	**0.71** (0.66-0.76)	**0.97 (**0.96-0.99)	**0.73** (0.69-0.78)	**0.97** (0.96-0.98)	**0.70** (0.65-0.75)	**0.97** (0.95-0.99)	**0.77** (0.71-0.83)
712	1576	710	1569	586	1389	372	875

[1] 'Enjoyment of food' was split on the median of the scores residualised for age at BEBQ completion and sex ($\leq$0.2982 and >0.2982).
[2] 'Enjoyment of food' was split on the median of the scores residualised for age at BEBQ completion, sex and gestational age ($\leq$0.2577 and >0.2577).
[3] Number of twin pairs.

Appendix 5.10. Sensitivity analyses for 'enjoyment of food' as a dichotomous variable

Data (N)	Model	Additive Genetic Effect (a^2)	Shared Environment Effect (c^2)	Non-shared Environment Effect* (e^2)	-2LL	df	Reference Model	Δ AIC	Δ BIC	$\Delta\chi^2$ (df)	*P*
1	**Sat**	-	-	-	5317.747	4575	-	-		-	-
(4581)	**ACE**	**0.53 (0.43-0.63)**	**0.45 (0.35-0.54)**	**0.03 (0.02-0.04)**	**5324.116**	**4578**	**Sat**	**0.369**	**-8.422**	**6.369 (3)**	**0.095**
	CE	-	0.82 (0.79-0.85)	0.18 (0.15-0.21)	5433.235	4579	1	107.120	50.691	109.120(1)	<0.001
	AE	0.98 (0.96-0.99)	-	0.02 (0.01-0.04)	5383.001	4579	1	56.886	25.574	58.886(1)	<0.001
	E	-	-	1.00 (1.00-1.00)	6350.604	4580	1	1022.488	505.507	1026.488(2)	<0.001
2	**Sat**	-	-	-	5263.023	4557	-	-		-	-
(4563)	**ACE**	**0.47 (0.38-0.57)**	**0.50 (0.40-0.58)**	**0.03 (0.02-0.05)**	**5274.229**	**4560**	**Sat**	**5.206**	**-5.998**	**11.206 (3)**	**0.011**
	CE	-	0.83 (0.80-0.86)	0.17 (0.14-0.20)	5367.892	4561	1	91.663	42.965	93.663(1)	<0.001
	AE	0.98 (0.96-0.99)	-	0.02 (0.01-0.04)	5349.121	4561	1	72.892	33.579	74.891(1)	<0.001
	E	-	-	1.00 (1.00-1.00)	6325.650	4562	1	1047.422	517.977	1051.422(2)	<0.001
3	**Sat**	-	-	-	4615.290	3949	-	-		-	-
(3955)	**ACE**	**0.54 (0.44-0.66)**	**0.43 (0.32-0.53)**	**0.03 (0.01-0.05)**	**4622.383**	**3952**	**Sat**	**1.093**	**-7.839**	**7.093 (3)**	**0.069**
	CE	-	0.81 (0.78-0.84)	0.19 (0.16-0.22)	4718.307	3953	1	93.924	44.166	95.924 (1)	<0.001
	AE	0.98 (0.96-0.99)	-	0.02 (0.01-0.04)	4669.575	3953	1	45.191	19.8	47.191 (1)	<0.001
	E	-	-	1.00 (1.00-1.00)	5482.187	3954	1	855.804	422.311	859.804 (2)	<0.001
4	**Sat**	-	-	-	3299.627	2818	-	-		-	-
(2824)	**ACE**	**0.43 (0.31-0.56)**	**0.54 (0.42-0.65)**	**0.03 (0.01-0.06)**	**3300.964**	**2821**	**Sat**	**-4.663**	**-10.377**	**1.337 (3)**	**0.720**
	CE	-	0.84 (0.80-0.87)	0.16 (0.13-0.20)	3345.509	2822	1	42.545	18.591	44.545(1)	<0.001
	AE	0.98 (0.96-0.99)	-	0.02 (0.01-0.04)	3352.786	2822	1	49.822	22.23	51.822(1)	<0.001
	E	-	-	1.00 (1.00-1.00)	3886.688	2823	1	581.724	285.499	585.724(2)	<0.001

Abbreviations: -2LL, -2 log likelihood; df, degrees of freedom; Reference Model, the model that the goodness of fit statistics are compared to; ΔAIC, change in Aikaike's Information Criterion statistic; ΔBIC, change in Bayesian Information Criterion statistic; $\Delta\chi^2$(df), change in Chi-square statistic (degrees of freedom); *P*, significance value.

Data: 1, includes all twins and scores are adjusted for age at BEBQ completion and sex; 2, includes all twins and scores are adjusted for age at BEBQ completion, sex and gestational age; 3, excludes twin pairs born before 34 weeks gestation, and scores are adjusted for age at BEBQ completion and sex; 4, excludes infants with any reported feeding problem, and scores are adjusted for age at BEBQ completion and sex.

Model: CE, AE and E models are nested within the full ACE model. The ACE model dissects the phenotypic variance into a^2, c^2 and e^2; the CE model drops the a^2 parameter and assesses the variance explained by the c^2 and e^2 parameters only; the AE model drops the c^2 parameter and assesses the variance explained by the a^2 and e^2 parameters only; the E model drops both the a^2 and c^2 parameters and assesses the variance explained by e^2 only. The most parsimonious model for each analysis is bolded.

Appendix 5.11. Goodness of fit statistics and parameter estimates for 'slowness in eating' as a dichotomous variable

Model	Additive Genetic Effect (a^2)	Shared Environment Effect (c^2)	Non-shared Environment Effect* (e^2)	-2LL	df	ΔAIC	ΔBIC	$\Delta\chi^2$ (df)	*P*
ACE	**0.66 (0.54-0.78)**	**0.29 (0.17-0.40)**	**0.05 (0.03-0.08)**	**5575.662**	**4606**	**-**	**-**	**-**	**-**
CE	-	0.75 (0.71-0.78)	0.25 (0.22-0.29)	5682.313	4607	104.652	49.454	106.652(1)	<0.001
AE	0.96 (0.93-0.97)	-	0.04 (0.03-0.07)	5596.735	4607	19.073	6.665	21.073(1)	<0.001
E	-	-	1.00 (1.00-1.00)	6389.430	4608	809.769	399.141	813.769 (2)	<0.001

Abbreviations: -2LL, -2 log likelihood; df, degrees of freedom; ΔAIC, change in Aikaike's Information Criterion statistic; ΔBIC, change in Bayesian Information Criterion statistic; $\Delta\chi^2$(df), change in Chi-square statistic (degrees of freedom); *P*, significance value.

Model: CE, AE and E models are nested within the full ACE model. The ACE model dissects the phenotypic variance into a^2, c^2 and e^2; the CE model drops the a^2 parameter and assesses the variance explained by the c^2 and e^2 parameters only; the AE model drops the c^2 parameter and assesses the variance explained by the a^2 and e^2 parameters only; the E model drops both the a^2 and c^2 parameters and assesses the variance explained by e^2 only. The most parsimonious model is bolded.

Appendix 5.12. Goodness of fit statistics for sex-limitation models for BEBQ scales

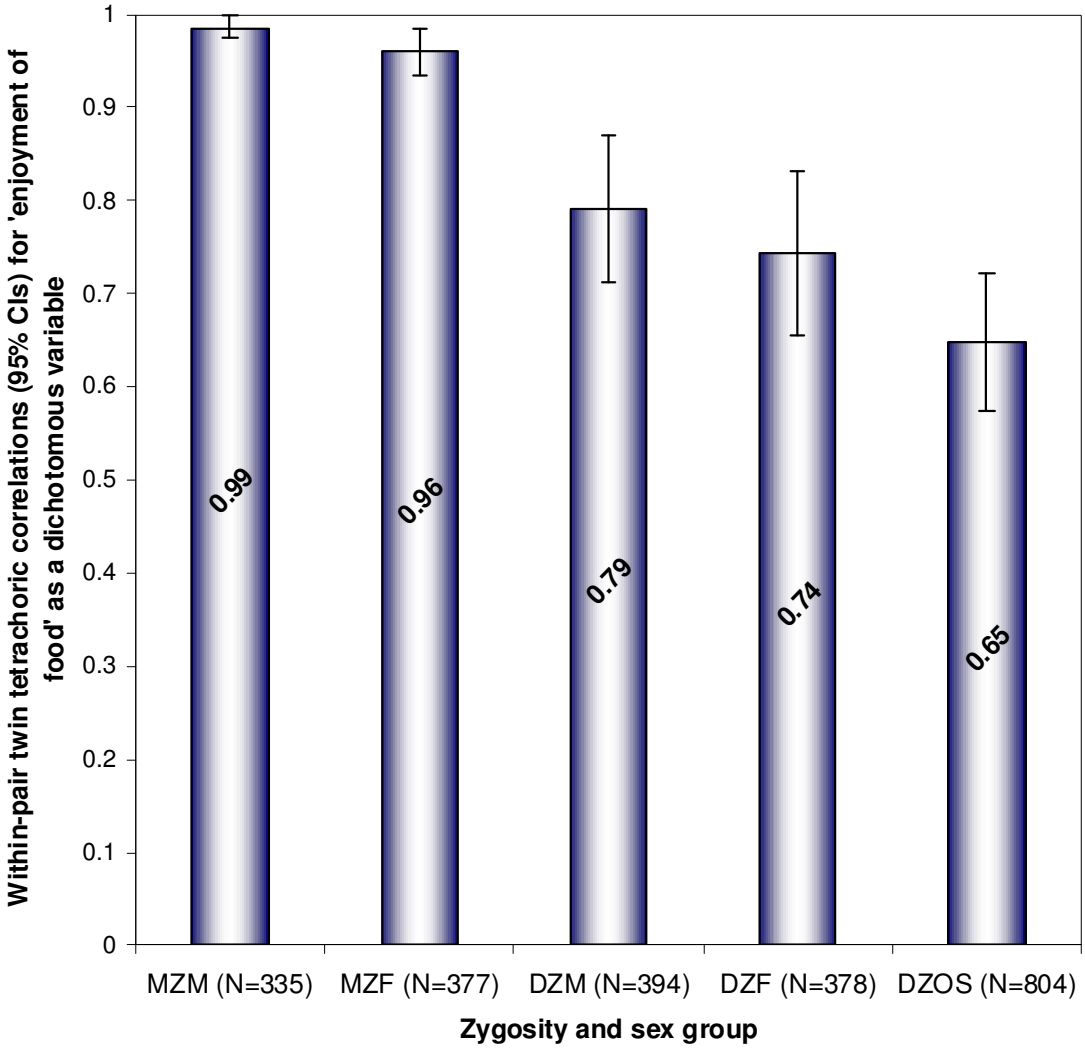
BEBQ Scale	Model	-2LL	df	Reference Model*	$\Delta\chi^2$ (df)	*P*	Δ AIC	Δ BIC
EF	**1. Saturated model**	11808.505	4557	-	-	-	-	
	2. Full sex-limitation model (r_A free)	11856.616	4570	1	48.111 (13)	<0.001	22.111	-26.239
	3. Full sex-limitation model (r_C free)	11861.196	4570	1	52.691 (13)	<0.001	26.691	-23.949
	4. Common effects model	**11861.196**	**4571**	**2**	**4.579 (1)**	**0.032**	**2.579**	**-1.579**
				3	0.00 (1)	1.00	-2.000	-3.869
	5. Null model	11877.585	4575	2	20.968 (5)	0.001	10.968	-8.86
				3	16.389 (5)	0.006	6.389	-11.15
FR	**1. Saturated model**	11141.866	4563	-	-	-	-	
	2. Full sex-limitation model (r_A free)	11162.055	4576	1	20.189 (13)	0.091	-5.811	-40.205
	3. Full sex-limitation model (r_C free)	11162.055	4576	1	20.189 (13)	0.091	-5.811	-40.205
	4. Common effects model	**11162.682**	**4577**	**2/3**	**0.627 (1)**	**0.428**	**-1.373**	**-3.556**
	5. Null model	11234.908	4581	2/3	72.853 (5)	<0.001	62.853	17.08
SE	**1. Saturated model**	11856.996	4585	-	-	-	-	
	2. Full sex-limitation model (r_A free)	11873.074	4598	1	16.078 (13)	0.245	-9.922	-42.295
	3. Full sex-limitation model (r_C free)	11873.074	4598	1	16.078 (13)	0.245	-9.922	-42.295
	4. Common effects model	11873.074	4599	2/3	0.000 (1)	1.000	-2.000	-3.871
	5. Null model	**11884.309**	**4603**	**2/3**	**11.235 (5)**	**0.047**	**1.235**	**-13.741**
SR	**1. Saturated model**	11705.743	4579	-	-	-	-	
	2. Full sex-limitation model (rA free)	11712.810	4592	1	7.067 (13)	0.899	-18.933	-46.789
	3. Full sex-limitation model (rC free)	11712.810	4592	1	7.067 (13)	0.899	-18.933	-46.789
	4. Common effects model	11714.327	4593	2/3	1.517 (1)	0.218	-0.483	-3.113
	5. Null model	**11732.626**	**4597**	**2/3**	**19.816 (5)**	**0.001**	**9.816**	**-9.447**
AS	**1. Saturated model**	12107.455	4590	-	-	-	-	-
	2. Full sex-limitation model (rA free)	12141.821	4603	1	34.366 (13)	0.001	8.366	-33.157
	3. Full sex-limitation model (rC free)	12141.821	4603	1	34.366 (13)	0.001	8.366	-33.157
	4. Common effects model	12141.821	4604	2/3	0.000 (1)	*	-2.000	-3.872
	5. Null model	**12163.418**	**4608**	**2/3**	**21.597 (5)**	**0.001**	**11.597**	**-8.563**

Abbreviations: BEBQ, Baby Eating Behaviour Questionnaire; EF, 'enjoyment of food'; FR, 'food responsiveness'; SE, 'slowness in eating'; SR, 'satiety responsiveness'; AS, 'appetite size'; -2LL, -2 log likelihood; df, degrees of freedom; Reference Model, the model that the goodness of fit statistics are compared to;$\Delta\chi^2$(df), change in Chi-square statistic (degrees of freedom); P, significance value, ΔAIC, change in Aikaike's Information Criterion statistic; ΔBIC, change in Bayesian Information Criterion statistic.

Models: 1, the saturated model contains the maximum number of free parameters to describe the data for each subgroup; 2, Full Sex-Limitation Model in which the additive genetic correlation (r_A) is estimated freely but the shared environment correlation (r_C) is fixed at 1.00 for opposite-sex dizygotic twin pairs, and ACE parameters are estimated separately for males and females; 3, Full Sex-Limitation Model in which r_C is estimated freely but r_A is fixed at 0.5 for opposite-sex dizygotic twin pairs, and ACE parameters are estimated separately for males and females; 4, Common Effects Model, r_A is fixed at 0.5 and r_C is fixed at 1.00 for opposite-sex dizygotic twin pairs, and ACE parameters are estimated separately for males and females; 4, Null Model, all parameter estimates are equated for males and females. The best-fitting model is bolded.

*For FR, SE, SR and AS both of the full sex-limitation models (Model 2 and Model 3) had the same fit statistics so subsequent more constrained models are only nested within the model that allows the genetic correlation to vary. For EF the two sex-limitation models had different fit statistics so the more constrained sub-models are nested within each.

Appendix 5.13. Within-pair tetrachoric correlations (and 95% confidence intervals) for 'enjoyment of food' as a dichotomous variable by all combinations of zygosity and sex



Abbreviations: MZM, monozygotic male twins; MZF, monozygotic female twins; DZM, dizygotic male twins; DZF, dizygotic female twins; DZOS, dizygotic opposite-sex twins.

Appendix 5.14. Goodness of fit statistics for sex-limitation models for 'enjoyment of food' as a dichotomous variable

Model	-2LL	df	Reference Model	$\Delta\chi^2$ (df)	*P*	Δ AIC	Δ BIC
1. Saturated model	5299.119	4563	-	-	-	-	
2. Full sex-limitation model (rA free)	5312.612	4574	1	13.493 (11)	0.262	-8.507	-35.81
3. Full sex-limitation model (rA free)	5312.612	4574	1	13.493 (11)	0.262	-8.507	-35.81
4. Common effects model	5317.916	4575	2/3	5.304 (1)	0.021	3.304	-1.217
6. Null model	**5324.116**	**4579**	**2/3**	**11.504 (5)**	**0.042**	**1.504**	**-13.593**

Abbreviations: -2LL, -2 log likelihood; df, degrees of freedom; Reference Model, the model that the goodness of fit statistics are compared to; $\Delta\chi^2$(df), change in Chi-square statistic (degrees of freedom); *P*, significance value, ΔAIC, change in Aikaike's Information Criterion statistic; ΔBIC, change in Bayesian Information Criterion statistic.

Models: 1, the saturated model contains the maximum number of free parameters to describe the data for each subgroup; 2, Full Sex-Limitation Model in which the additive genetic correlation (r_A) is estimated freely but the shared environment correlation (r_C) is fixed at 1.00 for opposite-sex dizygotic twin pairs, and ACE parameters are estimated separately for males and females; 3, Full Sex-Limitation Model in which r_C is estimated freely but r_A is fixed at 0.5 for opposite-sex dizygotic twin pairs, and ACE parameters are estimated separately for males and females; 4, Common Effects Model, r_A is fixed at 0.5 and r_C is fixed at 1.00 for opposite-sex dizygotic twin pairs, and ACE parameters are estimated separately for males and females; 4, Null Model, all parameter estimates are equated for males and females. The best-fitting model is bolded.

Appendix 5.15. Parameter estimates (95% confidence intervals) for sex-limitation model-fitting for 'enjoyment of food' as a dichotomous variable

Model	Male parameter estimates			Female parameter estimates			r_A	r_c
	a^2_m	c^2_m	e^2_m	a^2_f	c^2_f	e^2_f		
Full sex-limitation model (r_A free)	0.38 (0.24-0.56)	0.60 (0.43-0.74)	0.02 (0.01-0.04)	0.43 (0.26-0.64)	0.53 (0.33-0.69)	0.04 (0.02-0.07)	0.20 (0.00, 0.46)	1.00
Full sex-limitation model (r_C free)	0.38 (0.24-0.56)	0.60 (0.43-0.74)	0.02 (0.01-0.04)	0.43 (0.26-0.64)	0.53 (0.33-0.69)	0.04 (0.02-0.07)	0.50	0.79 (0.77,0.96)
Common effects model	0.40 (0.24-0.65)	0.59 (0.34-0.74)	0.02 (0.01-0.04)	0.63 (0.38-0.76)	0.33 (0.21-0.58)	0.04 (0.02-0.07)	0.50	1.00

	Parameter estimates for sexes combined				
	a^2	c^2	e^2		
Null model	**0.53 (0.43-0.63)**	**0.45 (0.35-0.54)**	**0.03 (0.02-0.04)**	**0.50**	**1.00**

Abbreviations: a^2_m, c^2_m, e^2_m, additive genetic, shared environmental and non-shared environmental estimates for males, respectively; a^2_f, c^2_f, e^2_f, additive genetic, shared environmental and non-shared environmental estimates for females, respectively; a^2, c^2, e^2, additive genetic, shared environmental and non-shared environmental estimates respectively for males and females combined; r_A, genetic correlation between opposite-sex dizygotic twin pairs; r_C, shared environmental correlation between opposite-sex dizygotic twin pairs.

Models (the best-fitting model is bolded):
Full Sex-Limitation Model (r_A free) – the additive genetic correlation (r_A) is estimated freely but the shared environment correlation (r_C) is fixed at 1.00 for opposite-sex dizygotic twin pairs, and ACE parameters are estimated separately for males and females.
Full Sex-Limitation Model (r_C free) – the shared environment correlation (r_C) is estimated freely but the additive genetic correlation (r_A) is fixed at 0.5 for opposite-sex dizygotic twin pairs, and ACE parameters are estimated separately for males and females.
Common Effects Model – r_A is fixed at 0.5 and r_C is fixed at 1.00 for opposite-sex dizygotic twin pairs, and ACE parameters are estimated separately for males and females.
Null Model – all parameter estimates are equated for males and females.

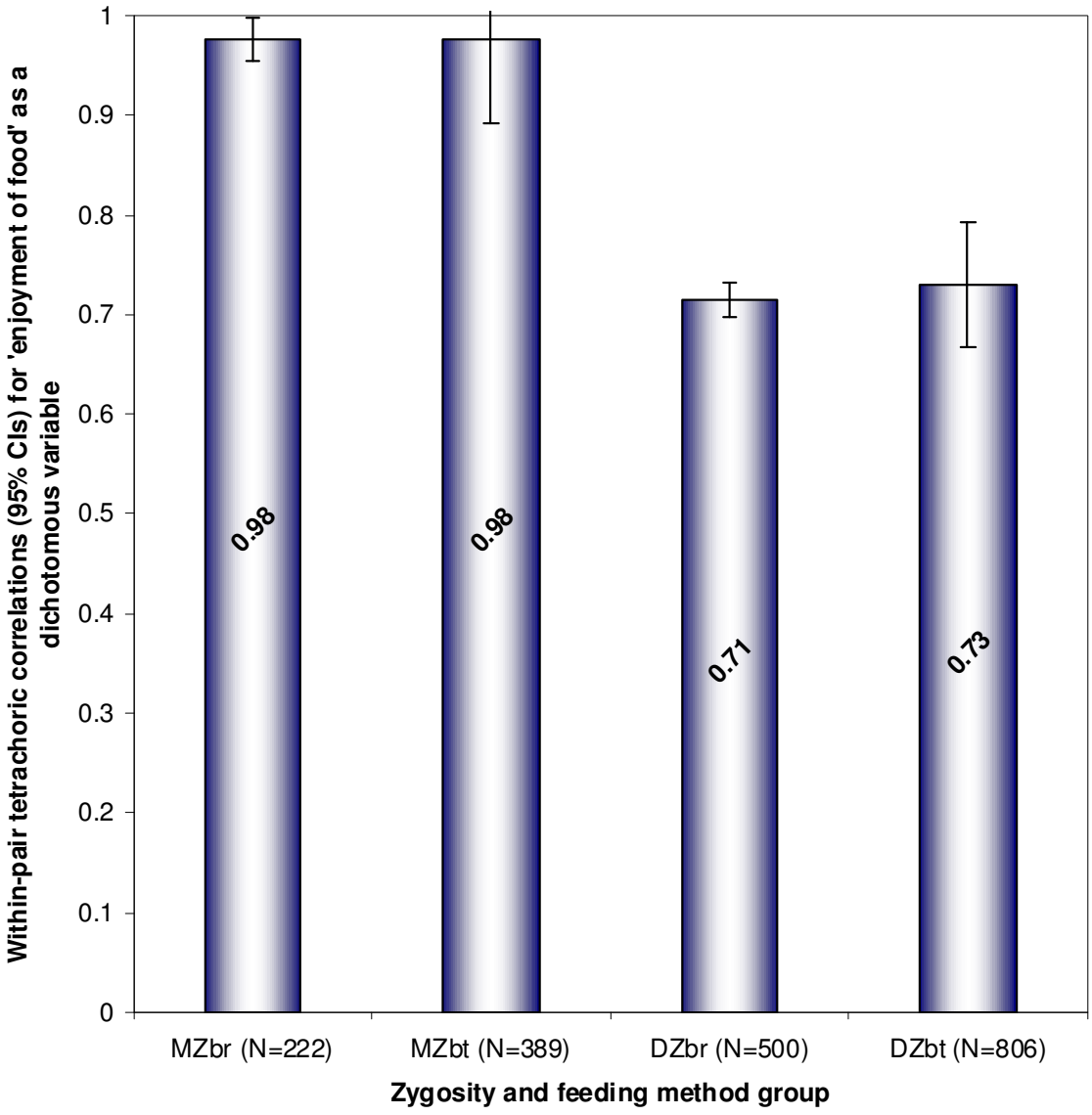
Appendix 5.16. Goodness of fit statistics for feeding method interaction models for BEBQ scales

BEBQ Scale (*n*)	Model	-2LL	df	Reference Model	$\Delta\chi^2$ (df)	*P*	Δ AIC	Δ BIC
EF	**1. Saturated model**	9732.085	3818	-	-	-	-	-
(3838)	**2. Common effects model**	9758.683	3828	1	26.598 (10)	0.003	6.598	-24.504
	3. Null model	**9772.161**	**3832**	**2**	**13.478 (4)**	**0.009**	**5.478**	**-8.383**
FR	**1. Saturated model**	9330.112	3826	-	-	-	-	-
(3846)	**2. Common effects model**	**9342.970**	**3836**	**1**	**12.858 (10)**	**0.232**	**-7.142**	**-31.382**
	3. Null model	9361.921	3840	2	18.951 (4)	0.001	10.951	-5.649
SE	**1. Saturated model**	9869.300	3844	-	-	-	-	-
(3864)	**2. Common effects model**	**9890.194**	**3854**	**1**	**20.894 (10)**	**0.022**	**0.894**	**-27.388**
	3. Null model	9928.010	3858	2	37.816 (4)	<0.001	29.816	3.775
SR	**1. Saturated model**	9625.392	3835	-	-	-	-	-
(3855)	**2. Common effects model**	9643.964	3845	**1**	**18.572 (10)**	**0.046**	**-1.428**	**-28.537**
	3. Null model	**9676.908**	**3849**	2	32.943 (4)	<0.001	24.943	1.342
AS	**1. Saturated model**	10072.597	3844	-	-	-	-	-
(3864)	**2. Common effects model**	10081.957	3854	1	9.360 (10)	0.498	-10.640	-33.156
	3. Null model	**10081.369**	**3858**	**2**	**5.412 (4)**	**0.248**	**-2.588**	**-12.429**

Abbreviations: BEBQ, Baby Eating Behaviour Questionnaire; EF, 'enjoyment of food'; FR, 'food responsiveness'; SE, 'slowness in eating'; SR, 'satiety responsiveness'; AS, 'appetite size'; -2LL, -2 log likelihood; df, degrees of freedom; Reference Model, the model that the goodness of fit statistics are compared to; $\Delta\chi^2$(df), change in Chi-square statistic (degrees of freedom); *P*, significance value; ΔAIC, change in Aikaike's Information Criterion statistic; ΔBIC, change in Bayesian Information Criterion statistic.

Models: 1, the saturated model contains the maximum number of free parameters to describe the data for each subgroup; 2, Common Effects Model, ACE parameters estimated separately for breast-fed and bottle-fed infants; 3, Null Model, all parameter estimates are equated for breast-fed and bottle-fed infants. The best-fitting model is bolded.

Appendix 5.17. Within-pair tetrachoric correlations (and 95% confidence intervals) for 'enjoyment of food' as a dichotomous variable by all combinations of zygosity and feeding method



Abbreviations: MZbr, breast-fed monozygotic twins; MZbt, bottle-fed monozygotic twins; DZbr, breast-fed dizygotic twins; DZbt, bottle-fed dizygotic twins.

Appendix 5.18. Goodness of fit statistics for feeding method interaction models for 'enjoyment of food' as a dichotomous variable

Model	-2LL	df	Reference Model	$\Delta\chi^2$ (df)	*P*	Δ AIC	Δ BIC
1. Saturated model	4409.832	3826	-	-	-	-	-
2. Common effects model	4417.784	3832	1	7.952 (6)	0.242	-4.048	-18.706
3. Null model	**4424.042**	**3836**	**2**	**6.258 (4)**	**0.181**	**-1.742**	**-11.992**

Abbreviations: -2LL, -2 log likelihood; df, degrees of freedom; Reference Model, the model that the goodness of fit statistics are compared to; $\Delta\chi^2$(df), change in Chi-square statistic (degrees of freedom); *P*, significance value; ΔAIC, change in Aikaike's Information Criterion statistic; ΔBIC, change in Bayesian Information Criterion statistic.

Models: 1, the saturated model contains the maximum number of free parameters to describe the data for each subgroup; 2, Common Effects Model, ACE parameters estimated separately for breast-fed and bottle-fed infants; 3, Null Model, all parameter estimates are equated for breast-fed and bottle-fed infants. The best-fitting model is bolded.

Appendix 5.19. Parameter estimates (95% confidence intervals) for feeding method interaction model-fitting for 'enjoyment of food' as a dichotomous variable

Common Effects Model						Null Model		
Breast-feeding parameter estimates			Bottle-feeding parameter estimates			Feeding methods combined		
a^2_{br}	c^2_{br}	e^2_{br}	a^2_{bt}	c^2_{bt}	e^2_{bt}	a^2	c^2	e^2
0.53 (0.37-0.71)	0.45 (0.27-0.60)	0.02 (0.01-0.06)	0.49 (0.36-0.63)	0.49 (0.35-0.61)	0.03 (0.01-0.05)	**0.50 (0.40-0.61)**	**0.47 (0.37-0.57)**	**0.03 (0.01-0.04)**

Abbreviations: a^2_{br}, c^2_{br}, e^2_{br}, additive genetic, shared environmental and non-shared environmental estimates respectively for breast-fed infants; a^2_{bt}, c^2_{bt}, e^2_{bt}, additive genetic, shared environmental and non-shared environmental estimates respectively bottle-fed infants; a^2, c^2, e^2, additive genetic, shared environmental and non-shared environmental estimates respectively for bottle-fed and breast-fed infants combined.

Models (the best-fitting model is bolded):
Common Effects Model – ACE parameters are estimated separately for breast-fed and bottle-fed infants.
Null Model – all parameter estimates are equated for breast-fed and bottle-fed infants.

Appendix 6. Additional table for Chapter 9 (Shared pathways underlying appetitive traits in infants)

Appendix 6.1. Goodness of fit statistics for multivariate models for BEBQ scales (*n*=4754)

Model	-2LL	df	Reference Model	$\Delta\chi^2$ (df)	*P*	Δ AIC	Δ BIC
1. Saturated	32145.357	13739	-	-	-	-	-
2. ACE: CFM	**32203.118**	**13763**	**1**	**57.761 (24)**	**<0.001**	**9.761**	**-64.07**
3. AE[1]	32316.657	13769	2	113.539 (6)	<0.001	101.539	33.532
4. CE[2]	33330.305	13769	2	1127.187 (6)	<0.001	1115.187	540.356
5. E[3]	36501.039	13775	2	4297.921 (12)	<0.001	4273.921	2102.485
6. ACE/rA,rE[4]	**32221.113**	**13766**	**2**	**17.995 (3)**	**<0.001**	**11.995**	**-2.622**
7. ACE/rC,rE[5]	32430.666	13766	2	227.548 (3)	<0.001	221.548	102.155
8. ACE/rA,rC[6]	33019.610	13766	2	816.492 (3)	<0.001	810.492	396.627
9. ACE/rA[7]	33072.105	13769	2	868.987 (6)	<0.001	856.987	411.256
10. ACE/rC[8]	34627.618	13769	2	2424.500 (6)	<0.001	2412.500	1189.012
11. ACE/rE[9]	32732.918	13769	2	529.800 (6)	<0.001	517.800	241.662
12. AE/rA,rE[10]	32221.113	13767	2	17.995 (4)	0.001	9.995	-6.494
13. AE/rA,rE[11]	**32225.489**	**13768**	**2**	**22.371 (5)**	**<0.001**	**12.371**	**-8.179**
			1	**80.132 (29)**	**<0.001**	**22.132**	**-72.249**
14. ACE: CPM	**32239.493**	**13767**	**1**	**94.136 (28)**	**<0.001**	**38.136**	**-61.374**
			2	36.375 (4)	<0.001	28.375	2.696
15. AEs/ACEc[12]	32334.424	13770	14	94.931 (3)	<0.001	88.931	35.846
16. ACEs/AEc[13]	**32239.493**	**13768**	**14**	**0.000 (1)**	*	**-2.000**	**-3.873**
17. AEs/AEc[14]	32334.424	13771	14	94.931 (4)	<0.001	86.931	31.973
18. CEs/ACEc[15]	33068.726	13770	14	829.233 (3)	<0.001	823.233	402.997
19. ACEs/CEc[16]	32451.672	13768	14	212.179 (1)	<0.001	210.179	102.216
20. CEs/CEc[17]	33352.880	13771	14	1113.386 (4)	<0.001	1105.386	541.201
21. Es/ACEc[18]	35509.522	13773	14	3270.029 (6)	<0.001	3258.029	1611.776
22. ACEs/Ec[19]	32732.918	13769	14	493.425 (2)	<0.001	489.425	238.966
23. Es/Ec[20]	36501.039	13775	14	4261.546 (8)	<0.001	4245.546	2099.789
24. AEs/AEc[21]	32239.515	13769	14	0.022 (2)	0.989	-3.978	-3.862
25. AEs/AEc[22]	**32243.319**	**13770**	**14**	**3.825 (3)**	**0.281**	**- 2.175**	**-9.706**
			1	**97.962 (31)**	**<0.001**	**35.962**	**-71.08**
			2	**40.201 (7)**	**<0.001**	**26.201**	**-7.01**
			13	**17.830 (2)**	**<0.001**	**13.830**	**1.382**

Statistical abbreviations: -2LL, -2 log likelihood; df, degrees of freedom; Reference Model, the model that the goodness of fit statistics are compared to;$\Delta\chi^2$(df), change in Chi-square statistic (degrees of freedom); *P*, significance value; ΔAIC, change in Aikaike's Information Criterion statistic; ΔBIC, change in Bayesian Information Criterion statistic; * probability incalculable because of no change in fit statistics.

Model name abbreviations: CFM, Correlated Factors Model; CPM, Common Pathway Model

Sub-models of the Correlated Factors Model:
[1] Drops the shared environment parameters.
[2] Drops the additive genetic parameters.
[3] Drops the shared environment and additive genetic parameters.
[4] Drops the shared environment correlations.
[5] Drops the additive genetic correlations.
[6] Drops the unique environment correlations.
[7] Drops the shared and unique environment correlations.
[8] Drops the additive genetic and unique environment correlations.
[9] Drops the additive genetic and shared environment correlations.
[10] Drops the shared environment parameter for 'enjoyment of food' only, and drops all of the shared environment correlations.
[11] Drops the shared environment parameters for 'enjoyment of food' and 'slowness in eating', and drops all of the shared environment correlations.

Sub-models of the Common Pathway Model:
[12] Drops the specific shared environment parameters, but retains the common environment parameter(s).
[13] Drops the common shared environment parameter, but retains the specific shared environment parameters.
[14] Drops the common shared environment parameter and also drops the specific shared environment parameters.
[15] Drops the specific additive genetic parameters, but retains the common additive genetic parameter.
[16] Drops the common additive genetic parameter, but retains the specific additive genetic parameters.
[17] Drops the common additive genetic parameter and also drops the specific additive genetic parameters.
[18] Drops the specific additive genetic parameters and also drops the specific shared environment parameters but keeps the common additive genetic and shared environment parameters.
[19] Drops the common additive genetic and shared environment parameters but keeps the specific additive genetic and shared environment parameters.
[20] Drops the common additive genetic and shared environment parameters and also drops the specific additive genetic and shared environment parameters.
[21] Drops the common shared environment parameter and also drops the specific shared environment parameter for 'enjoyment of food'.
[22] Drops the common shared environment parameter and also drops the specific shared environment parameters for 'enjoyment of food' and 'slowness in eating'.

Appendix 7. Additional tables for Chapter 10 (Shared pathways underlying appetite and weight in infants)

Appendix 7.1. Within-pair intraclass correlations (95% confidence intervals) for 3-mth weight SDS: all twins, with additional adjustment for gestational-age, without 'premature' infants, and without 'problem-feeders'

Intraclass Correlations (95% Confidence Intervals)							
n[1]							
All twin pairs[2]		With adjustment for gestational age[3]		Excluding infants born < 34 weeks[4]		Excluding 'problem-feeders'[4]	
MZs	DZs	MZs	DZs	MZs	DZs	MZs	DZs
0.67 (0.63-0.71)	**0.48** (0.44-0.52)	**0.67** (0.63-0.71)	**0.48** (0.44-0.52)	**0.69** (0.64-0.73)	**0.49** (0.45-0.54)	**0.73** (0.68-0.78)	**0.50** (0.45-0.55)
635	1406	635	1406	524	1235	352	870

[1] *n* refers to the number of twin pairs.
[2] Within-pair correlations using 3-mth weight SD scores regressed on sex and exact age (months and days) when the weight measure was taken.
[3] Within-pair correlations using 3-mth weight SD scores regressed on sex, exact age (months and days) when the weight measure was taken, and gestational age in weeks.
[4] Within-pair correlations using 3-mth weight SD scores regressed on sex and exact age (months and days) when the weight measure was taken.

Appendix 7.2. Sensitivity analyses for 3-month weight SDS

Data (*n*)	Model	Additive Genetic Effect (a^2)	Shared Environment Effect (c^2)	Non-shared Environment Effect (e^2)	-2LL	df	Reference Model	$\Delta\chi^2$ (df)	*P*	Δ AIC	Δ BIC
1	**Sat**	-	-	-	10848.484	4081	-	-	-	-	
(4214)	**ACE**	**0.37 (0.27-0.47)**	**0.30 (0.21-0.38)**	**0.33 (0.30-0.37)**	**10851.883**	**4086**	**Sat**	**3.399 (5)**	**0.639**	**-6.601**	**-17.364**
	CE	-	0.54 (0.51-0.57)	0.46 (0.43-0.49)	10896.289	4087	1	44.406 (1)	<0.001	42.406	18.39
	AE	0.70 (0.66-0.73)	-	0.30 (0.28-0.34)	10892.587	4087	1	40.704 (1)	<0.001	38.704	16.539
	E	-	-	1.00 (1.00-1.00)	11597.319	4088	1	745.435 (2)	<0.001	741.435	365.092
2	**Sat**	-	-	-	10851.453	4081	-	-	-	-	
(4214)	**ACE**	**0.37 (0.27-0.48)**	**0.29 (0.21-0.37)**	**0.33 (0.30-0.38)**	**10854.832**	**4086**	**Sat**	**3.379 (5)**	**0.642**	**-6.621**	**-17.375**
	CE	-	0.54 (0.51-0.57)	0.46 (0.43-0.49)	10898.726	4087	1	43.894 (1)	<0.001	41.894	18.134
	AE	0.69 (0.66-0.72)	-	0.31 (0.28-0.34)	10895.314	4087	1	40.482 (1)	<0.001	38.482	16.428
	E	-	-	1.00 (1.00-1.00)	11595.391	4088	1	740.560 (2)	<0.001	736.560	362.654
3	**Sat**	-	-	-	9205.207	3516	-	-	-	-	
(3633)	**ACE**	**0.34 (0.23-0.45)**	**0.33 (0.24-0.42)**	**0.33 (0.29-0.37)**	**9212.799**	**3521**	**Sat**	**7.592 (5)**	**0.180**	**-2.408**	**-14.897**
	CE	-	0.56 (0.52-0.59)	0.44 (0.41-0.48)	9245.898	3522	1	33.098 (1)	<0.001	31.098	12.811
	AE	0.70 (0.67-0.73)	-	0.30 (0.27-0.33)	9259.038	3522	1	46.238 (1)	<0.001	44.238	19.381
	E	-	-	1.00 (1.00-1.00)	9896.496	3523	1	683.696 (2)	<0.001	679.696	334.372
4	**Sat**	-	-	-	6260.266	2474	-	-	-	-	
(2561)	**ACE**	**0.45 (0.33-0.57)**	**0.30 (0.19-0.40)**	**0.25 (0.22-0.30)**	**6266.847**	**2479**	**Sat**	**6.581 (5)**	**0.254**	**-3.419**	**-14.803**
	CE	-	0.59 (0.55-0.63)	0.41 (0.37-0.45)	6311.819	2480	1	44.971 (1)	<0.001	42.971	18.866
	AE	0.77 (0.73-0.80)	-	0.23 (0.20-0.27)	6294.515	2480	1	27.668 (1)	<0.001	25.668	10.215
	E	-	-	1.00 (1.00-1.00)	6795.485	2481	1	528.638 (2)	<0.001	524.638	257.081

Abbreviations: -2LL, -2 log likelihood; df, degrees of freedom; Reference Model, the model that the goodness of fit statistics are compared to; $\Delta\chi^2$(df), change in Chi-square statistic (degrees of freedom); *P*, significance value; ΔAIC, change in Aikaike's Information Criterion statistic; ΔBIC, change in Bayesian Information Criterion statistic.

Data: 1, includes all twins and scores are adjusted for exact age in days when the weight measure was taken; 2, includes all twins and scores are adjusted for exact age in days when the weight measure was taken, sex and gestational age in weeks; 3, excludes twin pairs born before 34 weeks gestation, and scores are adjusted for exact age in days when the weight measure was taken and sex; 4, excludes infants with any reported feeding problem, and scores are adjusted for exact age in days when the weight measure was taken and sex.

Model: CE, AE and E models are nested within the full ACE model. The ACE model dissects the phenotypic variance into a^2, c^2 and e^2; the CE model drops the a^2 parameter and assesses the variance explained by the c^2 and e^2 parameters only; the AE model drops the c^2 parameter and assesses the variance explained by the a^2 and e^2 parameters only; the E model drops both the a^2 and c^2 parameters and assesses the variance explained by e^2 only. The most parsimonious model for each analysis is bolded.

Appendix 7.3. Goodness of fit statistics for multivariate models for appetitive traits & 3-month weight SDS (*n*=4082)

Model	-2LL	df	Reference Model	$\Delta\chi^2$ (df)	*P*	Δ AIC	Δ BIC
1. Saturated Model	**41805.564**	**17731**	-	-	-	-	-
2. Correlated Factors Model	**41860.950**	**17773**	**1**	**55.366 (42)**	**0.081**	**-28.614**	**-134.87**
3. AE[1]	41993.994	17777	2	133.045 (4)	<0.001	125.045	51.04
4. CE[2]	42086.632	17777	2	225.683 (4)	<0.001	217.683	97.372
5. E[3]	42993.602	17781	2	1132.652 (8)	<0.001	1116.652	535.362
6. ACE/rA,rE[4]	**41906.282**	**17779**	**2**	**45.333 (6)**	**<0.001**	**33.333**	**-0.557**
7. ACE/rC,rE[5]	42071.976	17779	2	211.026 (6)	<0.001	199.026	82.29
8. ACE/rA,rC[6]	43175.539	17779	2	1314.590 (6)	<0.001	1302.590	634.071
9. ACE/rA[7]	43293.917	17785	2	1432.968 (12)	<0.001	1408.968	670.037
10. ACE/rC[8]	45681.614	17785	2	3820.665 (12)	<0.001	3796.665	1863.885
11. ACE/rE[9]	42228.692	17785	2	367.742 (12)	<0.001	343.742	137.424
12. Independent Pathway Model	**41917.888**	**17779**	**1**	**112.324 (48)**	**<0.001**	**16.324**	**-129.624**
			2		<0.001	44.938	5.246
13. Common Pathway Model	**42025.709**	**17785**	**1**	**220.145 (54)**	**<0.001**	**112.145**	**-98.937**
			2		<0.001	140.759	35.933
			12			95.821	30.687

Statistical abbreviations: -2LL, -2 log likelihood; df, degrees of freedom; Reference Model, the model that the goodness of fit statistics are compared to;$\Delta\chi^2$(df), change in Chi-square statistic (degrees of freedom); *P*, significance value; ΔAIC, change in Aikaike's Information Criterion statistic; ΔBIC, change in Bayesian Information Criterion statistic.

Sub-models of the Correlated Factors Model:

[1] Drops the shared environment parameters.
[2] Drops the additive genetic parameters.
[3] Drops the shared environment and additive genetic parameters.
[4] Drops the shared environment correlations.
[5] Drops the additive genetic correlations.
[6] Drops the unique environment correlations.
[7] Drops the shared and unique environment correlations.
[8] Drops the additive genetic and unique environment correlations.
[9] Drops the additive genetic and shared environment correlations.

Appendix 8. Papers that I have worked on during my PhD, and the conferences that I have presented at and attended

Appendix 8.1. Papers that I have worked on during my PhD

Published papers:

Llewellyn CH, van Jaarsveld CHM, Johnson L, Carnell S and Wardle, J. (2010). Nature and nurture in infant appetite: a twin analysis. *The American Journal of Clinical Nutrition, 91,* 1172-1179.

Llewellyn CH, van Jaarsveld CHM, Boniface D, Carnell S, and Wardle J. (2008). Eating rate is a heritable phenotype related to weight in children. *The American Journal of Clinical Nutrition, 88,* 1560-1566.

Llewellyn CH, Carnell S, Wardle J. Eating Behaviour and Weight in Children. (2010). In (Eds) L Moreno, I Pigeot, W Ahrens. *Epidemiology of Obesity in Children and Adolescents (Book I of II) – Prevalence and Aetiology.* Springer series; New York

Wardle J, **Llewellyn CH**, Sanderson S and Plomin R. (2009). The FTO gene and measured food intake in children. *International Journal of Obesity, 33,* 42-5.

Hill C, **Llewellyn CH**, Saxton J, Webber L, Semmler C, Carnell S, van Jaarsveld CHM, Boniface D and Wardle J. (2008). Adiposity and 'eating in the absence of hunger' in children. *International Journal of Obesity, 32,* 1499-1505.

Fisher A, van Jaarsveld CHM, **Llewellyn CH** and Wardle J. (2010). Environmental influences on children's physical activity: quantitative estimates using a genetically-sensitive design. *Public Library of Science ONE, 5,* e10110.

van Jaarsveld CHM, Johnson L, **Llewellyn CH**, Wardle J. (2010). Gemini: a UK twin birth cohort with a focus on early childhood weight trajectories, appetite and the family environment. *Twin Research and Human Genetics, 13,* 72-78.

Taylor N, Ashley L, Forster AS, **Llewellyn CH**, Lloyd G, Martin J, Vangeli E and Webber L. (2008). 'Health psychology in focus': A review of the 2008 postgraduate workshop. *Health Psychology Update, 17,* 35-49.

Papers under review:

Llewellyn CH, van Jaarsveld CHM, Johnson L, Carnell S and Wardle J. (2011). Development and factor structure of the Baby Eating Behaviour Questionnaire. *Under review with Appetite.*

Johnson L, **Llewellyn CH**, van Jaarsveld CHM, Cole T and Wardle J. (2011). Genetic and environmental influences on infant growth: prospective analysis of the Gemini twin birth cohort. *Under review with Pediatrics.*

Appendix 8.2. Conferences that I have presented at during my PhD

Llewellyn CH, van Jaarsveld CHM and Wardle J. Genetic influences on appetite over the developmental years. Presented a poster at *The Obesity Society*, San Diego, United States, in October 2010.

Llewellyn CH, van Jaarsveld CHM and Wardle J. Nature and nurture in infant appetite. Invited to give an oral presentation (delivered by a colleague) at the *International Congress on Obesity*, Stockholm, Sweden, July 2010.

Wardle J, **Llewellyn CH**, Sanderson S and Plomin R. The FTO gene and measured food intake in children. Presented a poster at the *Dresden Spring School* 'From vulnerability to resilience: Molecular genetic perspectives', Dresden, Germany, March 2010.

Llewellyn CH and Wardle J. Genetic influences on eating behaviours in children. Gave an oral presentation at the *British Psychological Society Division of Developmental Psychology Annual Conference*, Nottingham, UK, September 2009.

Llewellyn CH and Wardle J. Nature and nurture in infant and child appetite. Gave an oral presentation at the *Benjamin Franklin Lafayette Seminar Series*, Frejus, France, July 2009.

Llewellyn CH, van Jaarsveld CHM, Boniface D, Carnell S, and Wardle J. Eating rate is a heritable phenotype related to weight in children. Gave an oral presentation at the *European Congress on Obesity*, Geneva, Switzerland, May 2008.

Appendix 8.3. Other conferences and courses that I have attended during my PhD

ESRC Introduction to the Media for Early Career Researchers, September 24th 2009, at the Kensington Close Hotel, London.

MRC Social, Genetic and Developmental Psychiatry (SGDP) Centre Twin Model-Fitting Course at the 10th SGDP Summer School from 13th- 17th July 2009, at the Institute of Psychiatry, Kings College London.

The B**ritish** Re**search** A**nd** T**raining in** He**alth Psychology Initiative (BREATHE) workshop**, September 2008, entitled 'Health Psychology in Focus', including speakers Tim Anstiss, Neil Coulson and Mark Forshaw. At the time I was president of BREATHE and led the organization and running of the workshop.

Cumberland Lodge, January 2008. Three day conference for PhD students of the Epidemiology and Public Health department at UCL, entitled 'Perspectives on Risk' including speakers Sir Michael Marmot and Professor Robert West.

Appendix 9. Materials for the Baby Appetite and Baby Eating Study (BABES)

Appendix 9.1. BABES home visits protocol

Checklist before you go

Equipment

Camcorder
Tripod
2 DVDs – 1 to use and 1 spare
Pocket scales
Paediatric weighing scales
Infantometer
Skinfold thickness callipers
Tape measure
Height Measure
Body Composition Analyser
Countdown stopwatch

Participant Materials – make sure you have allocated a family ID number and written it on all materials before you give them to the participant!

1 feeding tasks sheet (for researcher during feeding tasks)
1 anthropometrics measurements sheet
1 participant information leaflet
2 x participant consent forms
2 x participant questionnaires (Questionnaire 1 & Questionnaire 2)
1 milk diary
1 milk diary instructions
A4 strong brown freepost envelope
A5 padded envelope (for scales, to go inside larger freepost envelope)
A copy of this protocol

When you arrive

1. Say hello to mum and talk her through the tasks for the visit, give her the information leaflet to read
2. Ask her to sign the consent form
3. Ask her where she usually feeds the baby so that you can set the camera and tripod up
4. Give her Questionnaire 1 to fill in while you set up the camera on the tripod – make sure that the light is shining from behind the camera on to the baby's face NOT behind the baby towards the camera (or it will be difficult to see the details of the baby's mouth for coding)
5. When mum has finished the questionnaire ask her to take the baby's nappy off to take some anthropometrics of the baby
   - Weight
   - Waist circumference
6. Mum then asked if she is happy to be measured – take:
   - Weight and body composition
   - Height
   - Waist circumference
7. Turn scales on and make sure they are set to the grams mode
8. Ask Mum to make up the bottles for the ad libitum milk feed – at least 2 – and weigh the empty bottle, bottle with power, bottle with powder and water (etc)
9. Fill in the details of the milk type for the ad libitum milk feed on the 'feeding tasks' sheet (for both bottles of milk)
10. Ask Mum to sit down in position to start feeding the baby, with scales close by
11. Set up countdown timer for 2 mins and turn on video cameras
12. Ask Mum to start feeding – start countdown timer as soon as bottle goes in to the mouth
13. Weigh bottle every 2 mins as countdown timer goes off – always reset countdown timer as soon as bottle goes in to baby's mouth again
14. Carry on until baby finished feeding
15. Set the countdown timer to 30 mins
16. Give Mum the feeding diary, freepost envelope and scales – talk her through the general questions and show her how to use the pocket scales
17. Ask mum to prepare the bottle of milk for the second feed – weigh empty bottle, bottle with powder, bottle with powder and water (if formula)
18. When countdown timer goes off get Mum to offer baby more milk and weigh bottle at the end
19. Take final measurements for baby - will need to take off some of the clothes for skinfold thicknesses
    - Length
    - Skinfold thicknesses – babies don't like this! If they get upset just leave them out
20. Thank mum and explain what she needs to do with the diary and Questionnaire 2, then go
21. Send a thank you email afterwards

Appendix 9.2. BABES ad libitum milk feed data collection sheet

Family ID number: _____ Born: First / Second

Ad Libitum Milk Feed

Pre-feed Info

Time now	
Time of last feed	
Type of milk (please delete as appropriate)	Breast/Formula
If formula milk, type used (please delete as appropriate)	Powdered/Concentrate/ Ready-made
If formula milk, brand & kind used	
Weight (grams): (1) empty bottle (2a) bottle with water AND bottle with water and formula power/concentrate (2b) bottle with ready-made formula/ breast milk	
Baby's mood (usual=1 unusual=5) **If baby's mood scored >1, please specify nature of the unusual mood**	1 2 3 4 5 (please circle) Happy / sleepy / irritable
Factors affecting baby's feeding: (1) Cold (2) Teething (3) Temperature (4) Colic (5) Reflux (6) Stomach upset (7) Large previous feed (8) Recent vaccination (9) Other	Yes/ No Yes/ No Yes/ No Yes/ No Yes/ No Yes/ No Yes/ No Yes/ No Please specify:

Additional Info if 2nd Bottle of Milk Offered

Type of milk (please delete as appropriate)	Breast/Formula
If formula milk, type used (please delete as appropriate)	Powdered/Concentrate/ Ready-made
If formula milk, brand & kind used	
Weight (grams): (1) empty bottle (2a) bottle with water AND bottle with water and formula power/concentrate (2b) bottle with ready-made formula/ breast milk	

AD-LIBITUM FEED INFO

Feed Interval	Weight of bottle with formula (grams)
2-mins	
4-mins	
6-mins	
8-mins	
10-mins	
12-mins	
14-mins	
16-mins	
18-mins	
20-mins	
22-mins	
24-mins	
26-mins	
28-mins	
30-mins	
32-mins	
34-mins	
36-mins	
38-mins	
40-mins	
42-mins	

44-mins	
46-mins	
48-mins	
50-mins	
52-mins	
54-mins	
56-mins	
58-mins	
60-mins	

Additional Milk Feed

Type of milk (please delete as appropriate)	Breast/Formula
If formula milk, type used (please delete as appropriate)	Powdered/Concentrate/ Ready-made
If formula milk, brand & kind used	
Weight (grams): (1) empty bottle (2a) bottle with water AND bottle with water and formula power/concentrate (2b) bottle with ready-made formula/ breast milk	
Weight of bottle with milk post-feed	

Appendix 9.3. BABES milk feeding diary

Family ID Number _____

Welcome to BABES – Baby Appetite and Baby Eating Behaviour Study

MILK DIARY

Health Behaviour Research Centre, Department of Epidemiology & Public Health, UCL, 1-19 Torrington Place, London, WC1E 6BT, c.llewellyn@ucl.ac.uk; 020 7679 1736

UCL

MILK DIARY CONTENTS PAGE

2

INSTRUCTIONS

3

MILK DIARY INSTRUCTIONS

We would like you to record in this diary all of the formula or breast milk your baby consumes for 3 days. Please choose 3 days that are convenient for you and your baby.

When to fill in the diary

Please try to record your baby's milk intake as you go, **not from memory** at the end of the day. Use written notes if you forget to take the diary with you.

Time spent in the care of others

If your baby spends time in the care of others during the recording period then we would very much appreciate it if those carers (e.g. other relatives, crèche staff, childminder, friend) would provide details of the milk consumed.

4

PLEASE COMPLETE THE "GENERAL QUESTIONS" ABOUT YOUR FEEDING METHODS *BEFORE* YOU FILL IN THE DIARY

In this section we will ask you to tell us about your *usual* feeding method (in PART A – "Usual Feeds") and any *other* feeding method (in PART B – "Other Feeds") that you sometimes use. For example:

- If you *usually* feed your baby a particular brand and type of formula milk (e.g. *Cow & Gate's "From Newborn" Powder*), but a different type of milk during the night time feed if your baby isn't sleeping well (e.g. *Cow & Gate's "Good Night Milk"),* we would like you to include the information about the usual feeds in PART A, and information about the occasional night time feeds in PART B.

- If you change the concentration of formula to milk for specific feeds (e.g. *adding 1 extra scoop of formula to the bottle for the night time feed*), we would like you to include the information about the concentration of formula to milk that you *usually* use in PART A, and the different concentration of formula to milk that you *sometimes* use, in PART B.

When you come to fill out the diary, you will be able to refer to the feeding information you provided in PARTS A & B, for each feed, letting us know whether you used your "usual" feeding method (PART A) or your "other" feeding method (PART B), under the "Type of Milk" heading.

It may be the case that for a particular feed, you end up using a different feeding method from either of the 2 methods that you will have told us about in PARTS A & B (e.g. *feeding your baby ready-made formula milk because you accidentally spilt the pre-prepared formula milk, and were out of the house*). If this is the case, you can select the option "exception", and give us details about this particular feed in the 3rd section of the "Additional Information", provided at the end of each diary day.

5

PLEASE PROVIDE THE FOLLOWING INFORMATION WHEN YOU FILL IN THE DIARY

Date and Time

Please write down the *date*, in the space provided, each time you start a new day of recording. Each time you feed your baby, please record *the time* at the beginning of each feed (as soon as your baby starts feeding), and at the end of each feed (as soon as your baby stops feeding), in the space provided.

Amount of Milk

How you provide information about the amount of milk your baby drinks at each feed will depend on whether you feed your baby directly from your breast, or through a bottle. The instructions for the two different methods are below:

✓ ***If you feed your baby milk (formula or breast) through a bottle:***

- *Before* you start feeding your baby, please weigh the bottle of milk that you are going to feed your baby (with the teat attached) on the electronic weighing scale provided. Please write the total weight, in grams, in the space provided in the diary.
- After the feed, please weigh the bottle (with any leftover milk in it), with the teat still attached. Write down the total weight in the space provided in the diary. It is important that you weigh the bottle after your baby has finished feeding, even if it looks empty.
- If you feed your baby a 2nd bottle of milk during the same feed, please repeat steps 1 & 2. Please write the additional weights *in the row beneath* the weights you wrote down for the 1st bottle, and put an asterisk before these weights (so that we know they refer to a 2nd bottle given during the same feed).

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- ✓ ***If you feed your baby breast milk fed directly from the breast:***
  Please simply note down the time when your baby starts breast-feeding, and the time when your baby finishes breast-feeding, and complete the "Milk Given". Leave the "Weight of Bottle", "Leftovers", "Milk Spilt" and "Teat" sections blank.

Type of Milk

For each feed, please tick one of the 3 available options provided under the "Milk Given" heading:

- ✓ Tick *"Usual"* if the type of milk and method used to feed your baby were the same as those you described in PART A of the "General Questions".
- ✓ Tick *"Other"* if the type of milk and method used to feed your baby were the same as those you described in PART B of the "General Questions".
- ✓ Tick *"Exception"* if the type of milk or method used to feed your baby were different to either of those you described in PARTS A & B of the "General Questions". Please give details about the method and type of milk fed to your baby during this feed in the 3rd section of the "Additional Information", provided at the end of each day.

Leftover or Spilt Milk

Each time your baby is fed milk (formula or breast) through a bottle, please indicate:

- ✓ Whether or not there was any milk left in the bottle at the end (even if it was a very small amount) by selecting "Yes" or "No" under the heading "Leftovers".
- ✓ Whether or not any milk was spilt during the feed, by selecting "Yes" or "No" under the heading "Milk Spilt".

If you fed your baby directly from the breast, please leave these sections blank.

7

Teat Flow & Size

Each time your baby is fed milk (formula or breast) through a bottle, please state the flow speed (e.g. "slow") and size (e.g. "small") of the teat that you used. If you don't know, please write "Unsure".

Was it a typical day?

After each day of recording, in an "Additional Information" section, we ask if: (1) there were any reasons why your baby fed more or less than usual, and (2) whether or not your baby vomited after any feeds.

Overleaf you can see an example of how the general questions, and one day of the diary, have been filled in. The example shows you how we would like you to record your baby's milk feeding information.

If you have any further questions after reading the instructions and the example, please contact Clare Llewellyn by telephone or email:
c.llewellyn@ucl.ac.uk**; 020 7679 1736**

Thank you for your time and effort – we really appreciate it!

8

EXAMPLE

9

GENERAL QUESTIONS ABOUT YOUR FEEDING METHODS
(Please complete these before you fill in the diary)

PART A: QUESTIONS ABOUT YOUR *USUAL* FEEDING METHOD

1. What is your *usual* method of feeding? *(please tick the appropriate box)*

(a) Formula milk, made up from powder/concentrate and water ✓
If you ticked (a) please complete ALL remaining questions in PART A

(b) Ready-made formula milk ☐
If you ticked (b) please complete question 2 below

(c) Expressed breast-milk through a bottle ☐
If you ticked (c) please go straight to PART B

(d) Breast milk fed directly from the breast ☐
If you ticked (d) please go straight to PART B

2. What brand, and type of formula milk do you *usually* use?

Brand: Cow & Gate

Type: From Newborn

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3. If you make the formula milk yourself, how much formula powder/concentrate and water do you *usually* use? *(Please include the household measure used to estimate this)*

Amount of Formula *(number of: scoops/ teaspoons/ dessertspoons/ grams)*	Amount of Water *(millilitres/ fluid ounces)*
7 scoops	*210 millilitres*

4. If you use scoops, teaspoons or dessertspoons to measure your formula, please indicate whether the measure is:

Heaped	Level	Less Than Level
☐	✓	☐

When you are filling in the diary, you will see that there is a heading called "Type of Milk", with 3 options available for you to tick: "Usual", "Other", or "Exception". If you have used the type of feeding method that you have just described here in PART A, please tick the column headed "Usual".

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PART B: QUESTIONS ABOUT *OTHER* FEEDING METHODS

5. Do you sometimes use a different method from the one described above to feed your baby?

☐ No ✓ Yes *If "Yes", please complete question 2 below*
If "No", please go straight to the diary

6. What other method do you use to feed your baby? *(If you use the same method as you stated in PART A, but simply change the amount of powder/concentrate and water that you use, please tick the same method as PART A and follow the instructions)*

(a)	Formula milk, made up from powder/concentrate and water *If you ticked (a) please complete ALL remaining questions in PART B*	✓
(b)	Ready-made formula milk *If you ticked (b) please complete question 7 below*	☐
(c)	Expressed breast-milk through a bottle *If you ticked (c) please go straight on to complete the diary*	☐
(d)	Breast milk fed directly from the breast *If you ticked (d) please go straight on the complete the diary*	☐

7. What brand, and type of formula milk do you use with your other feed(s)? *(If you use the same brand and type of formula as you stated in PART A, but simply change the amount of powder/concentrate and water that you use, please write "See Part A" for "Brand" and "Type", and complete Q 5 below to give the information about the different amounts).*

Brand: *Cow & Gate* Type: *Goodnight Milk*

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8. **If you make the formula milk yourself for the other feed(s), how much formula powder/ concentrate and water do you normally use?** *(Please include the measure used to estimate this)*

Amount of Formula *(number of: scoops/ teaspoons/ dessertspoons/ grams)*	Amount of Water *(millilitres/ fluid ounces)*
7 scoops	*200 millilitres*

9. **If you use scoops, teaspoons or dessertspoons to measure your formula, please indicate whether the measure is:**

Heaped ✓ Level ☐ Less Than Level ☐

When you are filling in the feeding diary, if you have used the type of feeding method that you have just described here in PART B, please tick the column headed *"Other"*.

If you have NOT used EITHER of the feeding methods that you have described above in Parts A & B, please tick the column headed *"Exception",* and describe the method and type of milk used in the 3rd section of the "Additional Information", provided at the end of each day.

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DAY 1

Date: *04/09/2008*

TIME		WEIGHT OF BOTTLE (g)		MILK GIVEN			LEFTOVERS		MILK SPILT		TEAT	
Time feed began	Time feed finished	Before feed (g)	After feed (g)	Usual	Other	Exception	Yes	No	Yes	No	Flow	Size
08:03	*08:31*	*414g*	*353g*	✓				✓		✓	*Fast*	*Small*
10:10	*10:23*	*450g*	*357g*	✓			✓			✓	*Variable*	*Small*
13:26	*13:49*	*405g*	*290g*	✓				✓	✓		*Fast*	*Small*
15:32	*15:45*	*398g*	*255g*	✓		✓	✓			✓	*Fast*	*Small*
18:06	*18:20*	*425g*	*301g*	✓				✓		✓	*Fast*	*Small*
19:29	*20:10*	*420g*	*271g*	✓				✓		✓	*Fast*	*Small*
22:38	*23:30*	*417g*	*360g*		✓		✓			✓	*Variable*	*Small*
06:20	*06:45*	*450g*	*270g*	✓				✓		✓	*Fast*	*Small*
06:45	*07:01*	**390g*	**352g*	✓			✓			✓	*Fast*	*Small*

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ADDITIONAL INFORMATION – DAY 1

1. **Was the amount of milk that your baby had today about what they usually have, less than usual, or more than usual?**

Yes, **usual** ☐ No, **less** than usual ☑ No, **more** than usual ☐

Please tell us why s/he had less than usual

She has a cold and a temperature

Please tell us why s/he had more than usual

2. **Did your baby vomit after a feed today?**

Yes ☐ No ☑

If "Yes", please state the number of feeds: _____

15

3. **Please provide any additional information about "exceptional" feeds today**

Time Feed Began	Milk Type		
	Breast (Expressed/ direct from breast)	**Ready Made Formula** (Brand; type)	**Formula from powder/concentrate** (brand; type; amount of formula & water*; size of measure**)
15:32		Brand: *Cow & Gate* Type: *From Newborn*	Brand: _____ Type: _____ Formula/Water*: _____ Size of measure**: **heaped/ level/ less than level**
		Brand: _____ Type: _____	Brand: _____ Type: _____ Formula/Water*: _____ Size of measure**: **heaped/ level/ less than level**
		Brand: _____ Type: _____	Brand: _____ Type: _____ Formula/Water*: _____ Size of measure**: **heaped/ level/ less than level**

** Please report the amount of formula in household measures of scoops, teaspoons, dessertspoons, or grams. Please report the amount of water in fluid ounces or millilitres.*

*** Please delete as appropriate.*

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GENERAL QUESTIONS

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GENERAL QUESTIONS ABOUT YOUR FEEDING METHODS
(Please complete these before you fill in the diary)

PART A: QUESTIONS ABOUT YOUR *USUAL* FEEDING METHOD

1. **What is your *usual* method of feeding?** *(please tick the appropriate box)*

   (a) Formula milk, made up from powder/concentrate and water ☐
   If you ticked (a) please complete ALL remaining questions in PART A

   (b) Ready-made formula milk ☐
   If you ticked (b) please complete question 2 below

   (c) Expressed breast-milk through a bottle ☐
   If you ticked (c) please go straight to PART B

   (d) Breast milk fed directly from the breast ☐
   If you ticked (d) please go straight to PART B

2. **What brand, and type of formula milk do you *usually* use?**

   Brand: _____

   Type: _____

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3. If you make the formula milk yourself, how much formula powder/concentrate and water do you *usually* use? *(Please include the household measure used to estimate this)*

Amount of Formula *(number of: scoops/ teaspoons/ dessertspoons/ grams)*	Amount of Water *(millilitres/ fluid ounces)*
_____	_____

4. If you use scoops, teaspoons or dessertspoons to measure your formula, please indicate whether the measure is:

Heaped	Level	Less Than Level
☐	☐	☐

When you are filling in the diary, you will see that there is a heading called "Type of Milk", with 3 options available for you to tick: "Usual", "Other", or "Exception". If you have used the type of feeding method that you have just described here in PART A, please tick the column headed "Usual".

PART B: QUESTIONS ABOUT *OTHER* FEEDING METHODS

5. Do you sometimes use a different method from the one described above to feed your baby?

☐ No ☐ Yes *If "Yes", please complete question 2 below*
If "No", please go straight to the diary

6. What other method do you use to feed your baby? *(If you use the same method as you stated in PART A, but simply change the amount of powder/concentrate and water that you use, please tick the same method as PART A and follow the instructions)*

(a) Formula milk, made up from powder/concentrate and water ☐
If you ticked (a) please complete ALL remaining questions in PART B

(b) Ready-made formula milk ☐
If you ticked (b) please complete question 7 below

(c) Expressed breast-milk through a bottle ☐
If you ticked (c) please go straight on to complete the diary

(d) Breast milk fed directly from the breast ☐
If you ticked (d) please go straight on the complete the diary

7. What brand, and type of formula milk do you use with your other feed(s)? *(If you use the same brand and type of formula as you stated in PART A, but simply change the amount of powder/concentrate and water that you use, please write "See Part A" for "Brand" and "Type", and complete Q 5 below to give the information about the different amounts).*

Brand: _____ Type: _____

8. **If you make the formula milk yourself for the other feed(s), how much formula powder/ concentrate and water do you normally use?** *(Please include the measure used to estimate this)*

Amount of Formula *(number of: scoops/ teaspoons/ dessertspoons/ grams)*	Amount of Water *(millilitres/ fluid ounces)*
_____	_____

9. **If you use scoops, teaspoons or dessertspoons to measure your formula, please indicate whether the measure is:**

Heaped ☐ Level ☐ Less Than Level ☐

When you are filling in the feeding diary, if you have used the type of feeding method that you have just described here in PART B, please tick the column headed *"Other"*.

If you have NOT used EITHER of the feeding methods that you have described above in Parts A & B, please tick the column headed *"Exception",* and describe the method and type of milk used in the 3rd section of the "Additional Information", provided at the end of each day.

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The Diary
DAY 1

23

DAY 1

Date:												
TIME		WEIGHT OF BOTTLE (g)		MILK GIVEN			LEFTOVERS		MILK SPILT		TEAT	
Time feed began	Time feed finished	Before feed (g)	After feed (g)	Usual	Other	Exception	Yes	No	Yes	No	Flow	Size

24

ADDITIONAL INFORMATION – DAY 1

1. **Was the amount of milk that your baby had today about what they usually have, less than usual, or more than usual?**

Yes, **usual** ☐ No, **less** than usual ☐ No, **more** than usual ☐

Please tell us why s/he had less than usual	Please tell us why s/he had more than usual

2. **Did your baby vomit after a feed today?**

Yes ☐ No ☐

If "Yes", please state the number of feeds: _____

25

3. Please provide any additional information about "exceptional" feeds today

Time Feed Began	Milk Type		
	Breast (Expressed/ direct from breast)	**Ready Made Formula** (Brand; type)	**Formula from powder/concentrate** (brand; type; amount of formula & water*; size of measure**)
		Brand: _____ Type: _____	Brand: _____ Type: _____ Formula/Water*: _____ Size of measure**: **heaped/ level/ less than level**
		Brand: _____ Type: _____	Brand: _____ Type: _____ Formula/Water*: _____ Size of measure**: **heaped/ level/ less than level**
		Brand: _____ Type: _____	Brand: _____ Type: _____ Formula/Water*: _____ Size of measure**: **heaped/ level/ less than level**

** Please report the amount of formula in household measures of scoops, teaspoons, dessertspoons, or grams. Please report the amount of water in fluid ounces or millilitres.*
*** Please delete as appropriate.*

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The Diary
DAY 2

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DAY 2

Date:												
TIME		WEIGHT OF BOTTLE (g)		MILK GIVEN			LEFTOVERS		MILK SPILT		TEAT	
Time feed began	Time feed finished	Before feed (g)	After feed (g)	Usual	Other	Exception	Yes	No	Yes	No	Flow	Size

28

ADDITIONAL INFORMATION – DAY 2

1. **Was the amount of milk that your baby had today about what they usually have, less than usual, or more than usual?**

Yes, **usual** ☐ No, **less** than usual ☐ No, **more** than usual ☐

Please tell us why s/he had less than usual

Please tell us why s/he had more than usual

2. **Did your baby vomit after a feed today?**

Yes ☐ No ☐

If "Yes", please state the number of feeds: _____

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3. Please provide any additional information about "exceptional" feeds today

Time Feed Began	Milk Type		
	Breast (Expressed/ direct from breast)	**Ready Made Formula** (Brand; type)	**Formula from powder/concentrate** (brand; type; amount of formula & water*; size of measure**)
		Brand: _____ Type: _____	Brand: _____ Type: _____ Formula/Water*: _____ Size of measure**: **heaped/ level/ less than level**
		Brand: _____ Type: _____	Brand: _____ Type: _____ Formula/Water*: _____ Size of measure**: **heaped/ level/ less than level**
		Brand: _____ Type: _____	Brand: _____ Type: _____ Formula/Water*: _____ Size of measure**: **heaped/ level/ less than level**

** Please report the amount of formula in household measures of scoops, teaspoons, dessertspoons, or grams. Please report the amount of water in fluid ounces or millilitres.*
*** Please delete as appropriate.*

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The Diary
DAY 3

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DAY 3

Date:												
TIME		WEIGHT OF BOTTLE (g)		MILK GIVEN			LEFTOVERS		MILK SPILT		TEAT	
Time feed began	Time feed finished	Before feed (g)	After feed (g)	Usual	Other	Exception	Yes	No	Yes	No	Flow	Size

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ADDITIONAL INFORMATION – DAY 3

1. **Was the amount of milk that your baby had today about what they usually have, less than usual, or more than usual?**

Yes, **usual** ☐ No, **less** than usual ☐ No, **more** than usual ☐

Please tell us why s/he had less than usual

Please tell us why s/he had more than usual

2. **Did your baby vomit after a feed today?**

Yes ☐ No ☐

If "Yes", please state the number of feeds: _____

33

3. Please provide any additional information about "exceptional" feeds today

Time Feed Began	Milk Type		
	Breast (Expressed/ direct from breast)	**Ready Made Formula** (Brand; type)	**Formula from powder/concentrate** (brand; type; amount of formula & water*; size of measure**)
		Brand: _____ Type: _____	Brand: _____ Type: _____ Formula/Water*: _____ Size of measure**: **heaped/ level/ less than level**
		Brand: _____ Type: _____	Brand: _____ Type: _____ Formula/Water*: _____ Size of measure**: **heaped/ level/ less than level**
		Brand: _____ Type: _____	Brand: _____ Type: _____ Formula/Water*: _____ Size of measure**: **heaped/ level/ less than level**

** Please report the amount of formula in household measures of scoops, teaspoons, dessertspoons, or grams. Please report the amount of water in fluid ounces or millilitres.*
*** Please delete as appropriate.*

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Thank you

for completing this diary.

BABES

UCL